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Author(s): Ellen D. Ketterson and Val Nolan Jr.

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HORMONES AND LIFE HISTORIES: AN INTEGRATIVE APPROACH

ELLEN D. KETTERSON AND VAL NOLAN JR.

Department of Biology, Indiana University, Bloomington, Indiana 47405

Abstract.—This article, which focuses on hormones and the diverse effects they have on behavior and physiology, raises evolutionary questions that hormonal studies appear especially well suited to address. These include the endocrine basis for life-history trade-offs, the role of hormones in adaptive alterations in social organization and mating systems, and whether natural selection acts on traits or organisms. The article also shows how phenotypic engineering by hormonal manipulations can reveal the evolutionary significance of phenotypic variation. By generating rare or novel phenotypes, we can attempt to determine the shape of fitness profiles in nature. To illustrate phenotypic engineering, we manipulated plasma testosterone in a free-living bird, the dark-eyed junco (*Junco hyemalis*), and measured the effects of the treatment on behavior, including allocation of time to mate attraction and parental behavior as well as use of space, and on physiology, including regulation of body mass, corticosterone, and molt. We compared treated males to controls for various correlates of fitness, including territory acquisition, mate acquisition, mate retention, physical condition of the mate, apparent reproductive success, extrapair fertilizations, and survival. The results to date appear to indicate that selection is relatively indifferent to a broad range of phenotypes, while extreme deviations from the norm are selected against.

The objective of this article is to describe the potential that studies of hormones have to increase understanding of the consequences of variation in behavior and physiology and thus understanding of the evolution of life histories, social organization, and mating systems. We begin by reviewing some basic facts about the action of one hormone, testosterone, in birds. Our aim is to illustrate a few principles, including (1) that hormones are secreted in response to stimuli that are generated both internally and externally, (2) that hormones have multiple targets and diverse effects, and (3) that the complexity of hormonal action leaves ample room for variation on which natural selection can act.

We then pose four interconnected questions that relate hormones to the evolutionary process and that provide fruitful avenues for research: (1) How might hormones form the mechanistic bases for trade-offs in life histories? (2) What is the role of hormones in the evolution of inter- and intraspecific variation in social organization and mating systems? (3) Does natural selection act on organisms or on traits? (4) What is the evolutionary significance of phenotypic variation in hormonally mediated traits? From the recent literature we give examples of studies that address or could be used to address each question.

Finally, we describe an experimental method that we call phenotypic engineering (Ketterson et al., in press) and show how we and others have used this method to address the fourth question, the evolutionary significance of phenotypic variation. Our model organism is a small passerine bird, the dark-eyed junco

(*Junco hyemalis*). We have manipulated plasma concentrations of testosterone in free-living male juncos in order to alter their behavioral and physiological phenotypes. Some of the changes we have induced are potentially beneficial, while others are potentially detrimental. Our objective has been to determine the overall effect these phenotypic changes have on fitness.

ABOUT TESTOSTERONE

Testosterone, like other hormones, is remarkable because it is effective in minute quantities and has such a wide array of behavioral, physiological, and morphological consequences. To an evolutionary biologist, testosterone may be one of the most interesting hormones because it is so intimately associated with reproduction and affects such a wide variety of traits. What is presented here is necessarily oversimplified. For general reviews of the action and effects of testosterone, see Adler (1981) and Knobil and Neill (1988); for treatment of testosterone in birds, see Nalbandanov (1976), Epple and Stetson (1980), Balthazart (1983), and Silver and Ball (1989).

Testosterone is a steroid hormone secreted by the testes in response to gonadotropins, which are produced by the anterior pituitary gland in response to stimulation by releasing hormones from the hypothalamus. The stimuli that trigger secretion of testosterone are diverse, for example, day length, aggressive encounters with conspecifics, or female sexual behavior (Wingfield and Moore 1988); and testosterone contributes to its own regulation by suppressing further secretion of releasing hormones and gonadotropins.

Testosterone enters the circulation and influences cellular activity at target tissues in the central nervous system (e.g., many nuclei in the brain; see Balthazart and Ball, in press) and in the periphery (e.g., gonads, syrinx, integument, musculature; fig. 1). Individuals may vary in responsiveness to environmental stimuli, releasing hormones and gonadotropins; rate of secretion of testosterone; half-life of testosterone in the circulation; density of receptors at the targets; affinity of receptors for the hormone; and activity of the enzymes responsible for metabolic conversion of the hormone to its active form. Thus, the potential for complex interactions is great, and these interactions are critical to a full understanding of integrated organismal function. Values for many of the regulatory variables (e.g., density of receptors) cannot be known for animals that remain alive in the field, although it is possible to flood these receptors with compounds that block hormonal action (antiandrogens) (Hegner and Wingfield 1987; Beletsky et al. 1990; Schwabl and Kriner 1991). Fortunately, plasma concentrations of testosterone in free-living animals can be determined relatively easily, and they have been shown to correlate with many functional aspects of behavior and physiology.

As for behavior of birds, testosterone affects male incubation and feeding of young (Silverin 1980; Hegner and Wingfield 1987; Oring et al. 1989; Ketterson et al., in press), vocalizations (Wada 1981, 1982, 1986; Arnold 1982; Gyger et al. 1988; Harding et al. 1988; Nowicki and Ball 1989; Ketterson et al., in press), and aggressiveness (Balthazart 1983; Wingfield et al. 1987, 1990; Archawaranon and

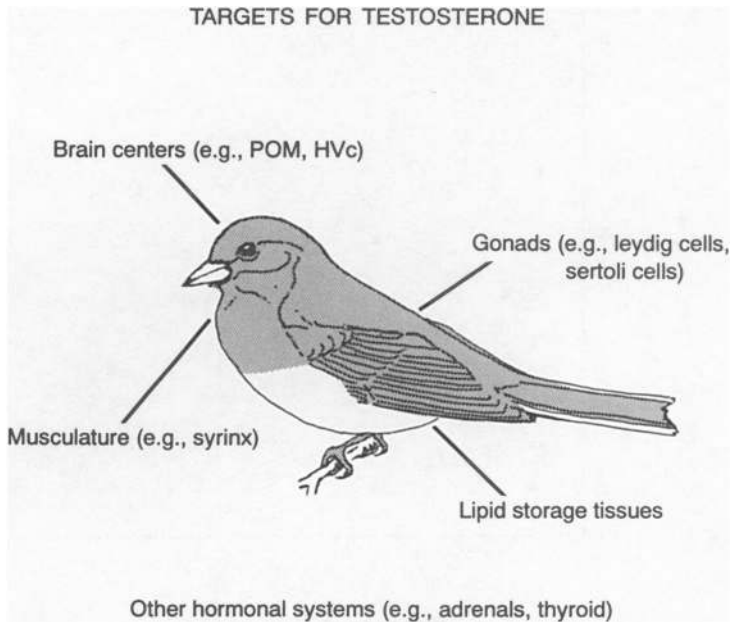


FIG. 1.—Targets for the hormone testosterone. Although the drawing is of a dark-eyed junco, the targets are taken from studies of a variety of bird species and have not necessarily been identified in the junco.

Wiley 1988; Beletsky et al. 1990). However, it is important to recall that the effect of steroids is not the direct production of behavior; rather, steroids affect the likelihood that behavior will be expressed (Feder 1984; Moore 1991). In terms of physiology, testosterone increases locomotor activity (Wada 1982, 1986; Massa and Bottoni 1987) and metabolic rate (Hännsler and Prinzinger 1979; Feuerbacher and Prinzinger 1981), and it suppresses lipid storage (Wingfield 1984; Ketterson et al. 1991a) and the onset of prebasic molt (Runfeldt and Wingfield 1985; Schleusner et al. 1985; Nolan et al. 1992). At the intraspecific level, very little is known about the quantitative relationship between natural variation in levels of testosterone and behavior. However, a recent study of male red-winged blackbirds (*Agelaius phoeniceus*) has shown that individual variation in testosterone correlates both with song rate and with frequency of aggressive interactions (Johnsen 1991) (fig. 2).

The question whether intraspecific variation in plasma testosterone has a genetic basis has received little study, but there is indirect evidence for an additive genetic component to variation in aggressiveness, which is influenced by testosterone (Boag 1982; Moss et al. 1982; Maxson et al. 1983). In quail (*Coturnix japonica*), artificial selection for high mating frequency, which is also associated with testosterone, leads to significant correlated responses in aggressiveness and in a morphological trait, the size of the cloacal gland (Sefton and Siegal 1975; Cunningham and Siegal 1978). Therefore, genetic variation for testosterone-

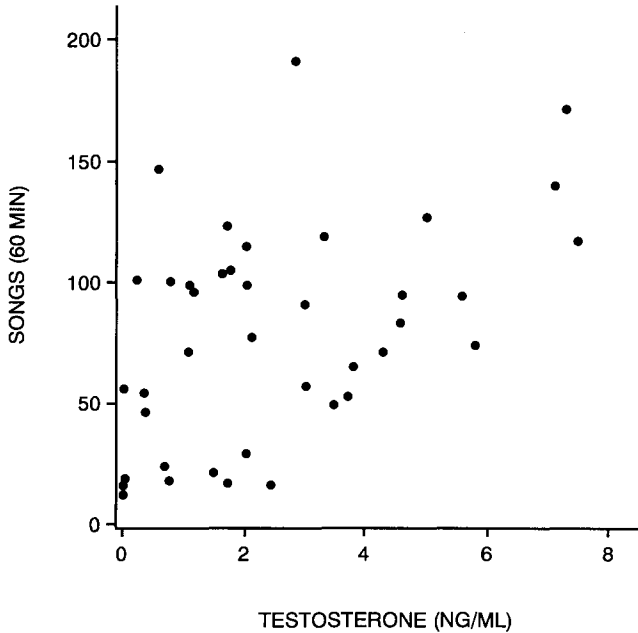


FIG. 2.—Song rate as a function of plasma concentration of testosterone in free-living red-winged blackbirds (*Agelaius phoeniceus*) observed during breeding. (From Johnsen 1991. Reprinted by permission.)

mediated traits seems likely. In the rest of this article, we will assume that such variation exists and that a response to selection is possible. Clearly, this point deserves further study.

HORMONES AND THE EVOLUTIONARY PROCESS

Question 1: How Might Hormones Form the Mechanistic Basis for Trade-offs in Life Histories?

According to Stearns (1989), trade-offs are fitness costs that occur when a beneficial change in one trait is linked to a detrimental change in another. Changes in fitness that occur when two traits depend on the same hormone and are co-expressed, but only one is beneficial, may also be regarded as trade-offs. In this view, hormones are one of the important mechanistic links between organismal biology and evolutionary ecology.

Despite the considerable attention that has been given to trade-offs (see reviews in Dingle and Hegmann 1982; Reznick 1985; Partridge and Harvey 1988; Stearns 1989), more effort has been devoted to measuring the cost of reproduction (De Steven 1980; Nur 1984) and documenting the genetic basis for variation in life-history traits (Dingle and Hegmann 1982) than to uncovering the physiological mechanisms that underlie trade-offs, particularly those associated with the endo-

crine system. Several investigators have recently called for more work in this area (Stearns 1989; West-Eberhard 1989, 1992; Ligon et al. 1990; Zuk et al. 1990).

The dearth of hormonal studies is surprising because hormones can produce correlated effects with potentially antagonistic fitness consequences, precisely the situation we seek when attempting to demonstrate the existence of trade-offs (Williams 1957). Thus, while it is obviously important to study genetic correlations among characters and to determine how these correlations vary across environments, it is equally important to study the proximate causes of genetic correlations. Only by knowing their physiological bases can we hope to understand how such correlations originate or how they can be disrupted by selection. Finally, the shortage of hormonal studies is surprising because, in terms of levels of organization, hormones lie between genes and life-history traits but are much easier to manipulate than genes.

Two recent examples of an experimental approach to hormones and life histories addressed the potential trade-offs between clutch size and egg size (Sinervo and Licht 1991a, 1991b) and between energy devoted to growth and energy devoted to maintenance (Derting 1989). Sinervo and Licht (1991a, 1991b) have studied egg size and egg number (clutch size) in female side-blotched lizards (*Uta stansburiana*). Females treated with follicle-stimulating hormone (FSH) produced clutches that were larger than normal (6.0 vs. 4.2 eggs), but their eggs were of smaller than normal size (0.33 vs. 0.43 g). In contrast, females whose follicles were partially ablated with a needle produced smaller than normal clutches, but their eggs were larger than normal. Variation in clutch size and variation in egg size in this species may be linked physiologically, and a major factor regulating both may be FSH. Tests are currently under way to compare the survival and fecundity of hatchling lizards produced from small and large eggs and then released into the field (B. Sinervo, personal communication).

Derting (1989) has investigated the potential for a trade-off between growth and maintenance in young cotton rats (*Sigmodon hispidus*). A basic assumption about animal life histories has been that energy is limiting and that energy devoted to maintenance is not available for production (growth and reproduction) (Cody 1966; Hirshfield and Tinkle 1975; van Noordwijk and de Jong 1986). However, Derting has shown that thyroid hormone can promote both production *and* maintenance and that the advantages of high or low thyroid activity may depend on environmental factors. She administered thyroid hormone (T_4) to young cotton rats in the laboratory. To one set of animals (A) she provided food ad lib.; in another set (R) food was restricted. Within each level of food, some subjects (E) were treated with T_4 to elevate their metabolic rate, while controls (N) were left untreated and had normal metabolism. The results (fig. 3) reveal a striking and unexpected interaction between maintenance (metabolic rate), production (growth), and food availability. Not surprisingly, the NA rats grew faster than the NR rats. However, the elevated EA rats grew even faster, while the ER rats barely grew at all. It is tempting to speculate, as Derting does, that food-rich environments might select for rats with greater thyroid activity, while other environments might favor animals with lower activity. The interesting outcome was that, given sufficient food, cotton rats treated with thyroid hormone apparently

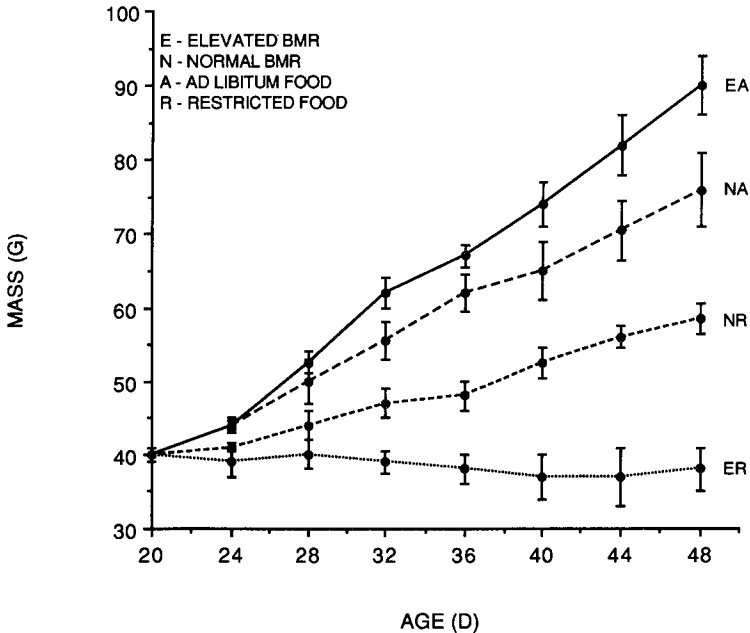


FIG. 3.—Growth of young cotton rats (*Sigmodon hispidus*). The four groups are EA, experimental animals treated with thyroid hormone (T_4) and given free access to food; ER, animals treated with T_4 whose access to food was restricted; NA, control animals not treated with T_4 and given free access to food; and NR, untreated control animals whose access to food was restricted. (From Derting 1989; © 1989 by the Ecological Society of America. Reprinted by permission.)

can increase production despite higher maintenance costs, thus avoiding the expected trade-off.

Moore (1991) has considered the role of hormones in the determination of alternative male strategies, another form of life-history trade-off. He drew a parallel between the organizational and activational effects of hormones during development and the decision rules employed by males for the adoption of alternative strategies. When strategies are fixed (males assume one form or another but do not shift, e.g., salmonid fishes) (Gross 1984, 1985), coordination of behavior and morphology is likely to be determined early in development through the organizing effects of hormones. When strategies are plastic and males may shift from one to another as the environment or their condition changes, switches of strategy are likely to be under activational control (e.g., sex change in the stoplight parrotfish, *Sparisoma viride*) (Cardwell and Liley 1991a, 1991b). Moore provides numerous examples of his interesting thesis and strongly urges an experimental hormonal approach to development and alternative male strategies (see also West-Eberhard 1992).

Collectively, the studies reviewed in this section suggest that hormones can provide mechanistic explanations for the existence of life-history trade-offs.

Question 2: What Is the Role of Hormones in the Evolution of Inter- and Intraspecific Variation in Social Organization and Mating Systems?

Several workers have recently asserted that variation in patterns of secretion of a few key hormones or variation in responsiveness of target tissues to these same hormones might explain much inter- and intraspecific variation in vertebrate social behavior, social organization, and mating systems. Because these efforts to relate hormones to social evolution are relatively new, of necessity they are somewhat speculative. As examples we refer to the work of Wingfield and his colleagues (1987, 1990), Boinski (1991), and Carter (in press).

It is widely known that males of polygynous bird species tend to provide less parental care than males of monogamous species (Verner and Willson 1969). (We use the terms *monogamous* and *polygynous* in the traditional sense to refer to species in which males form social pair bonds with one or more than one female.) It is also true that polygynous males, unlike males of most monogamous species, often continue to display after they have acquired their first mate (Emlen and Oring 1977; Nolan 1978; Vehrencamp and Bradbury 1984; Wingfield et al. 1990). At the physiological level, there is evidence to suggest that testosterone is at least partially responsible for this conflict between mating effort and parental effort (Wingfield et al. 1987, 1990).

Interspecific variation in patterns of testosterone secretion among birds correlates with interspecific variation in the mating system (Wingfield et al. 1987, 1990). Comparison of seasonal profiles of plasma testosterone of polygynous and monogamous species reveals that polygynous males tend to sustain secretion at peak levels for much of the breeding season, whereas monogamous males exhibit a single brief peak early in the breeding season (Wingfield et al. 1987). The ratio of summer to winter levels of testosterone is also significantly associated with mating system and parental behavior: the higher the ratio, the more likely a species is to be polygynous (Wingfield et al. 1990). However, an aggressive challenge (e.g., by an intruder) leads to a greater increase in testosterone in monogamous than in polygynous species (Wingfield 1991). Apparently testosterone of polygynous males is closer to the physiological maximum during most of the breeding season, while monogamous species maintain lower levels (of testosterone) but secrete higher levels "on demand."

Boinski (1991) has detected correspondence between geographical variation in social organization of free-living squirrel monkeys (*Saimiri* spp.) and geographical variation in adrenocortical function among laboratory-held *Saimiri*. Squirrel monkeys in Costa Rica and Peru differ in group size, levels of aggression, dominance relations between the sexes, and patterns of natal dispersal (Mitchell et al. 1991). Boinski speculates that the populations may also differ in disposition or emotional reactivity. The populations that are more aggressive (Peru) may have a higher setting to their adrenocortical axis, which would be reflected in higher levels of cortisol and slower recovery from episodes of stress. This could be the result of local adaptation or a few key (genetic?) changes in receptor density, availability of neurotransmitter, or levels of adrenocorticotrophic hormone. Boinski speculates further that this variation may be driven by geographical variation in spatial

distribution of the foods eaten by the monkeys. When the preferred food is abundant but concentrated (Peru), group size and social interaction increase; when food is dispersed (Costa Rica), social stress may be less a factor in driving social organization. This system is currently under study.

Finally, Carter (in press) has detected a relationship among small mammals in the frequency with which females nurse young and males initiate copulation, and she suspects that the pattern may be explained by a shared mechanism, the hormone oxytocin. Female rabbits nurse young once a day, and a single brief mating bout is sufficient to induce pregnancy. Female rats nurse several times a day, and mating activity lasts for several hours. Female prairie voles lactate continuously while in the nest, and matings may go on for more than a day. Only the prairie voles are monogamous. Carter speculates that oxytocin may regulate this pattern of interspecific covariation between the sexes in lactation frequency and duration of copulation. Oxytocin facilitates the muscular contractions that lead to milk release and ejaculation. It may also act in the brain to produce the sensation of satiety that terminates bouts of both lactation and sexual behavior and to promote social bonds that form between mother and young and, in some species, between male and female. If lactation, sexual behavior, and social bonding have a common physiological basis, natural selection acting on regulation of hormonal action could account for diverse aspects of interspecific differences in social organization. It could also account for correlated interspecific changes in the functioning of both males and females.

Although primarily correlative, these studies of hormonal action and social behavior are intriguing and point to the potential importance of hormones and behavior in the evolution of social systems. The overwhelming diversity in behavior, physiology, and morphology that characterizes vertebrates may ultimately be explicable in terms of a relatively small number of physiological systems.

Question 3: Does Selection Act on Organisms or Traits?

According to Travis (1989), the focus of selection is one of the central questions in evolutionary biology. When several traits are dependent on the same hormone, it seems likely that even if selection acts on traits, the effect will be to shape organisms. That is, if selection favors individuals with high values of a trait whose expression depends on hormonal concentration, then it will necessarily act on the other traits whose expression also depends on the same hormone. If changes in all traits are beneficial, we would expect individuals exhibiting favorable values to increase in frequency. But if some changes are advantageous while others are disadvantageous, then prediction becomes more difficult. Such negative correlations among traits might be viewed as design constraints that limit adaptation, or, as already seen, they may represent trade-offs that persist because they allow the organism to adjust its tactics to varying and unpredictable environmental variation.

Organisms also have the capacity to dissociate control of different hormone-dependent traits, that is, to compartmentalize their attributes and thus to insulate themselves against detrimental effects that might occur when selection favors only one among a complex of hormone-dependent traits. For example, modifica-

tion of the number or affinity of receptors at the different targets of the hormone could dissociate responses, which would thereby reduce detrimental effects of a change in hormonal concentration while enhancing beneficial effects. Similarly, a target may require that a hormone be converted from one form to another before it is active. If conversion were to depend on an inducible enzyme, this dependence on induction could account for loss of a trait or for condition-dependent expression. Finally, behaviors that were once hormone-dependent can become uncoupled from hormones altogether (e.g., copulatory behavior; see Moore and Marler 1988). In quail, testosterone activates both copulatory behavior and crowing (see Balthazart and Ball, in press), but the neuroanatomical sites for these behaviors are separated physically. Copulation (neck grab, mount, and cloacal contact movement) requires that testosterone be aromatized enzymatically into E_2 at the preoptic medial nucleus, while crowing depends on enzymatic reduction of testosterone to 5-alpha-dihydrotestosterone in the nucleus intercollicularis. According to Balthazart and Ball (in press), "Circulating steroids in the plasma provide a general signal for an increase in reproductive activity that is then modified and amplified at the various targets."

Many variables will determine the outcome of a situation in which selection favors one of a complex of hormone-dependent traits, and the answer to the question of whether selection ultimately acts on organisms or traits is almost certainly that it acts on both. Although this kind of analysis seems critical to an understanding of the evolution of integrated organismal function, few endocrine studies address this point directly in an evolutionary context. The studies of correlated responses to selection for aggressiveness or sexual behavior in quail (see About Testosterone, above) seem promising (Sefton and Siegal 1975; Cunningham and Siegal 1978). Such studies should provide ample work for physiologists to determine the multiple changes that might accompany a response to selection (e.g., plasma hormonal concentrations, receptor levels and affinities, activity levels of enzymes that convert hormones from one form to another, and alterations in cellular activities at the target).

Question 4: What Is the Evolutionary Significance of Intrapopulational Phenotypic Variation?

Phenotypic engineering as an experimental approach.—Phenotypic engineering consists of manipulating the phenotype of an organism, quantifying the effects of the manipulation, and relating these effects to performance or fitness (Ketterson et al., in press). This method permits exploration of the evolutionary significance of phenotypic variation by asking whether a rare or a novel phenotype would increase in frequency, assuming the requisite genetic variation (cf. Sinervo and Huey 1990).

The classical approach to understanding the consequences of variation in a trait is to modify it and determine the effect on fitness. An elegant example is the work of Andersson (1982). By varying tail length of male widowbirds (*Euplectes progne*), Andersson found that females prefer males with longer tails, even when the tails are longer than those that occur naturally. This raises the obvious question of what prevents the evolution of longer tails in male widowbirds, but the

answer is elusive because the classical approach has been to alter just a single trait and to measure just a few fitness consequences of such alterations. We cannot know whether, if males were to produce longer tails, they would also differ in other ways (traits as well as fitness consequences) that would be detrimental (see Selander's [1965] thoughts on the effect of tail length on survival of male grackles, *Quiscalda* spp.).

Phenotypic engineering with hormones.—Modifying phenotypes by varying circulating levels of hormones is of particular interest precisely because hormones influence so many interlocking traits. Thus, hormonal manipulations enable the investigator to avoid the trait-by-trait study of organisms and to measure the effect of simultaneous changes in suites of characters. At the risk of exaggerating the point by ignoring for the moment the capacities of organisms to compartmentalize their responses, the method provides the potential to predict the outcome of generations of direct and indirect selection, all in a single experiment.

Thus, by comparing the fitness of hormonally produced rare or novel phenotypes with the fitness of unaltered controls, it is possible to probe the question of why existing phenotypes persist despite the fact that alternative phenotypes are possible. In fact, without experimental intervention, relating variation in phenotype to variation in fitness is a difficult undertaking, in part because most traits are normally distributed and individuals that express extreme values of a trait are rare. Phenotypic engineering allows the investigator to create large numbers of extreme individuals and to see how they fare. At least one caveat seems important, however: the success of some phenotypes, particularly those involving social behavior, is likely to be frequency-dependent.

In the future, investigators will almost certainly use hormones to manipulate both single traits and suites of traits in order to comprehend integrated organismal responses to selection. Neurobiologists in the laboratory are able to implant microquantities of hormones in targeted portions of the brain in order to study localized effects of hormones on behavior. In theory it should also be possible to employ these techniques in the field in order to determine the fitness consequences of uncoupling traits as well as coupling them.

Phenotypic manipulations and correlates of fitness: possible outcomes.—How might hormonal manipulations affect fitness? Three possible outcomes are depicted in figure 4 (after Falconer 1989), which can be described as paradoxical, adaptive, and neutral. Curve 1, the paradoxical outcome, shows fitness as a positive linear function of the magnitude of a trait and indicates that individuals with high values perform better than those at or below the population mean; the paradox is that selection has not led to such higher values. The adaptive outcome is depicted in curve 2. Individuals that deviate from the mean in either direction have lower fitness than those at the mean. This is the situation we would expect under normalizing selection. In curve 3, fitness is relatively independent of the value of the trait; individuals that deviate widely from the norm are equally effective at leaving descendants. This outcome would imply that the character is not important to fitness; the model is compatible with the interpretation that fitness is largely determined by chance.

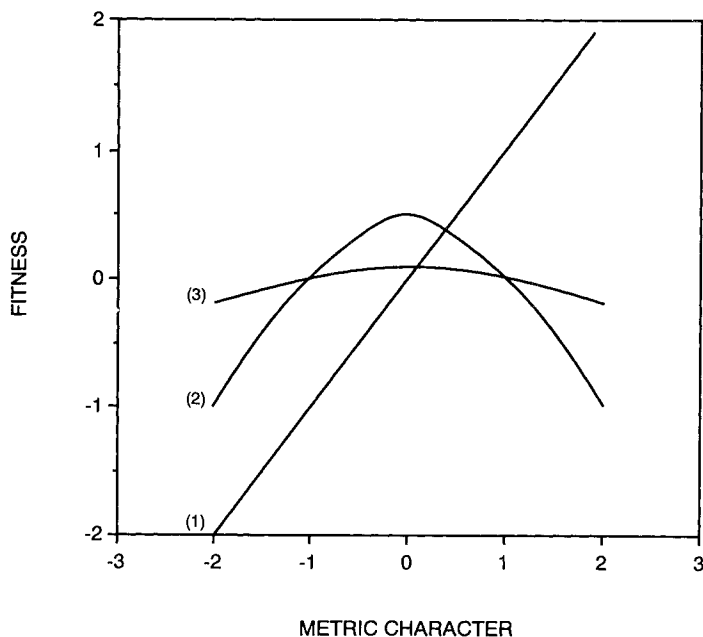


FIG. 4.—Fitness profiles showing possible relationships between variation in a quantitative trait and fitness. Numbers on the X-axis indicate SDs of the mean. (From Falconer 1989, *Quantitative Genetics*, © 1989 by John Wiley & Sons. Reprinted by permission.)

An example of this manipulative approach to the study of fitness is the work of Marler and Moore (1988a, 1988b, 1989, 1991) on the mountain spiny lizard, *Sceloporus jarrovi*. These investigators treated free-living male lizards with long-lasting implants of testosterone and observed their behavior, physiology, and survival rate. Testosterone-implanted males displayed more, spent more time above ground, and had higher field metabolic rates and lower energy reserves. They also suffered higher mortality than controls (fig. 5). It is not known whether the treatment affected their reproductive success.

Experimental studies of the effect of testosterone on fitness in free-living male birds have shown an influence on major components of fitness, namely acquisition of mates (Watson and Parr 1981; Wingfield 1984), maintenance of territory (Beletsky et al. 1990), production of offspring (Hegner and Wingfield 1987; Oring et al. 1989), and survival (Dufty 1989; Nolan et al. 1992). Some of the effects on fitness are negatively correlated—testosterone increases the number of mates acquired by song sparrows (*Melospiza melodia*) but decreases survival of brown-headed cowbirds (*Molothrus ater*). In the house sparrow (*Passer domesticus*), males treated with testosterone rear fewer offspring to fledging than do controls, because their mates are apparently unable to compensate for the reduction in male parental effort (Hegner and Wingfield 1987). Whether elevated testosterone would

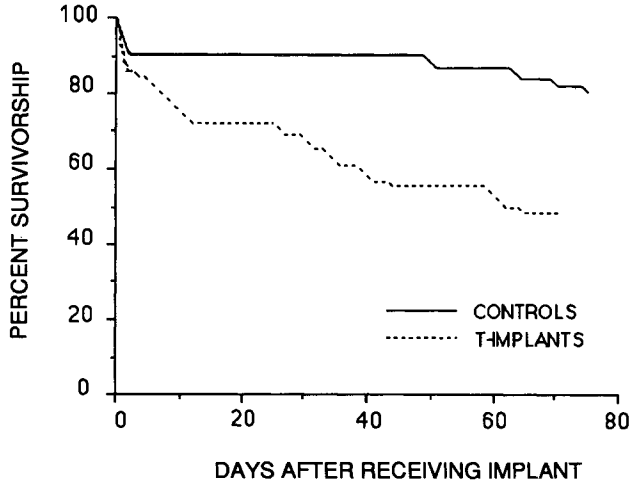


FIG. 5.—Summer survival rate in mountain spiny lizards (*Sceloporus jarrovi*), according to treatment. (From Marler and Moore 1988a. Reprinted by permission.)

also have beneficial effects on male house sparrows is not known, although Hegner and Wingfield (1987) speculate that males with high testosterone might be more successful in extrapair fertilizations.

PHENOTYPIC ENGINEERING IN JUNCOS: A CASE HISTORY

We made it our objective to try this approach in the field. We too manipulated testosterone and set out to quantify its phenotypic effects and to relate these changes to as many components of fitness as we were able to measure.

The Study Species

Dark-eyed juncos are members of the avian subfamily Emberizinae and are abundant and widely distributed in North America. The subspecies that we study, the Carolina junco (*Junco hyemalis carolinensis*), is an altitudinal migrant that breeds at high elevations in the Appalachian Mountains of southeastern North America. During the breeding season, males are territorial and form a pair-bond with a single female, although polygyny occasionally occurs (<10%; E. D. Ketterson and V. Nolan, personal observations). Female juncos build the nest, which is typically on the ground, and do all the incubation. Both sexes protect eggs, and both feed and protect nestlings and fledglings.

Our study area is the University of Virginia's Mountain Lake Biological Station in southwestern Virginia and its environs (see Wolf 1987 for a description). Our general field methods and demographic findings are summarized in Wolf et al. (1988, 1990, 1991), Ketterson et al. (1991a, 1991b, in press), and Nolan et al. (1992). Since 1983, we have color-marked over 3,000 individuals. Pairs tend to remain together for the entire breeding season, and fidelity to the previous year's

breeding site is high among adults of both sexes. When both pair members survive winter, they usually re-form the pair. Yearlings tend to breed close to their natal sites, and some young males fail to obtain territories and become floaters (Ketterson et al. 1991*b*). The breeding sex ratio is somewhat male biased.

Breeding begins in late April and continues until mid-August; clutch size ranges from three to five. Occasional pairs raise as many as three broods in a season, but nest predation in some years is very high (average across years = 66% loss between laying and fledging), so many pairs leave no offspring in a season. In the winter, juncos live in flocks. Adult males tend to remain near their breeding territories, while females and yearlings normally move downslope (Ketterson et al. 1991*b*).

Hormonal Treatments, Assays, and Rationale

We randomly assign males to treatment within two categories: age (first-year vs. older) and habitat (wet woods, dry woods, open areas). Thus, we study approximately equal numbers of experimental males (T-males) and controls (C-males) of each age and in each habitat.

Birds are implanted with two 10-mm lengths of Silastic tubing (Dow Corning; i.d. = 1.47 mm, o.d. = 1.96 mm) (Ketterson et al. 1991*a*), either packed with crystalline testosterone or left empty. To check the effectiveness of implants as well as to measure natural variation in circulating levels of testosterone, we recapture the birds after treatment, take a blood sample, and use radioimmunoassay to determine plasma concentrations of steroid hormones. Our assay methods are the same as those of Wingfield, as described in Wingfield and Farner (1975), Wingfield et al. (1982), and Ketterson et al. (1991*a*).

Time of implanting has varied from year to year (fig. 6). In one year (1989), we began implanting in early March, but in most years (1987, 1988, 1990), birds were treated in April and May. Implants were removed in August, except during 1990, when we delayed removal until October and in some males left implants in place all winter.

The rationale and effectiveness of our treatment can best be understood by comparing figures 6 and 7. Testosterone (T) ordinarily peaks in male juncos in early spring, after which it falls and is maintained at lower levels for the remainder of the breeding season (fig. 7; E. D. Ketterson and V. Nolan, unpublished observations in 1989), although males retain the capacity to increase T throughout the breeding season (data not shown). In males with T implants, T is elevated to peak physiological levels for at least 40 d, and in some individuals it remains higher than normal for an additional period of perhaps 100 d (Nolan et al. 1992). Thus, we have used our treatment (1) to maintain T at its peak for the entire breeding season (1987, 1988), (2) to raise T in early spring, before it peaks in the natural population (1989), and (3) to prolong a high level beyond the end of the breeding season (1990). The rationale for treatment 1 was to mimic the seasonal profile of a typical polygynous species (Wingfield et al. 1987). The rationale for manipulations 2 and 3 was to probe the factors that might set the limits on the timing of seasonal reproduction. Although we altered the seasonal pattern in these ways, we did not raise T above levels that juncos are capable of achieving naturally.

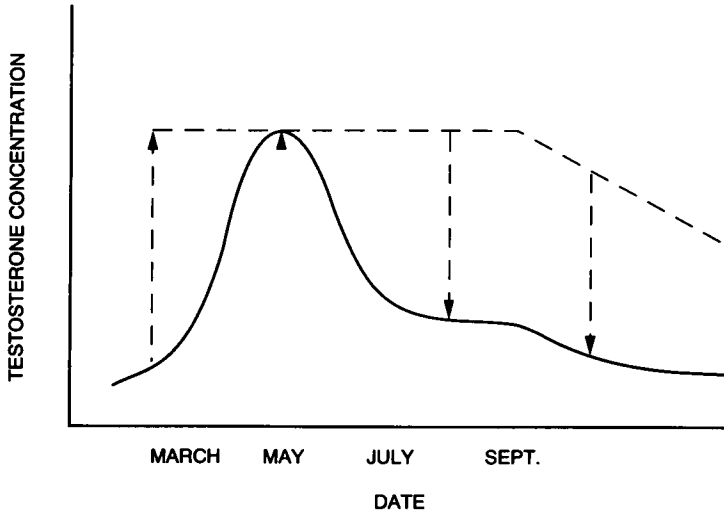


FIG. 6.—The *solid line* is a schematic diagram of the seasonal profile of plasma testosterone concentration in untreated male dark-eyed juncos. The *dashed lines and arrows* are also schematic and indicate the timing of insertion (*upward arrows*) and removal (*downward arrows*) of testosterone implants and their intended effects on plasma concentration of testosterone. The *dashed line* extending beyond October indicates the presumed testosterone level of males whose implants were not removed.

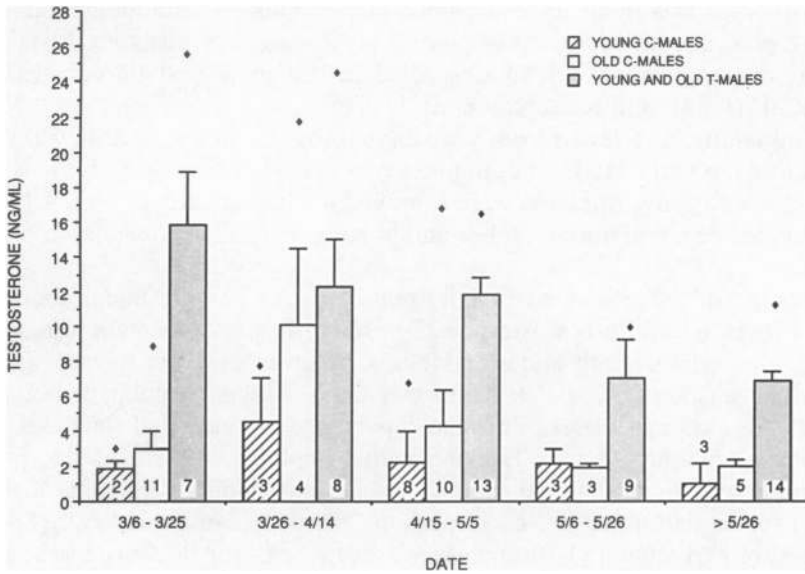


FIG. 7.—Seasonal profile of plasma concentration of testosterone in treated and untreated dark-eyed juncos (mean \pm 1 SE; *n* in histogram bar indicates size of sample). The untreated birds are classified according to their age. Young birds were in their first breeding season, and old birds were in their second or subsequent breeding season. *Diamonds* indicate the maximum value observed in each treatment group during each time period. Where no diamonds are plotted, the maximum lay within the SE bar (E. Ketterson and V. Nolan, unpublished data from 1989, when implanting began in March).

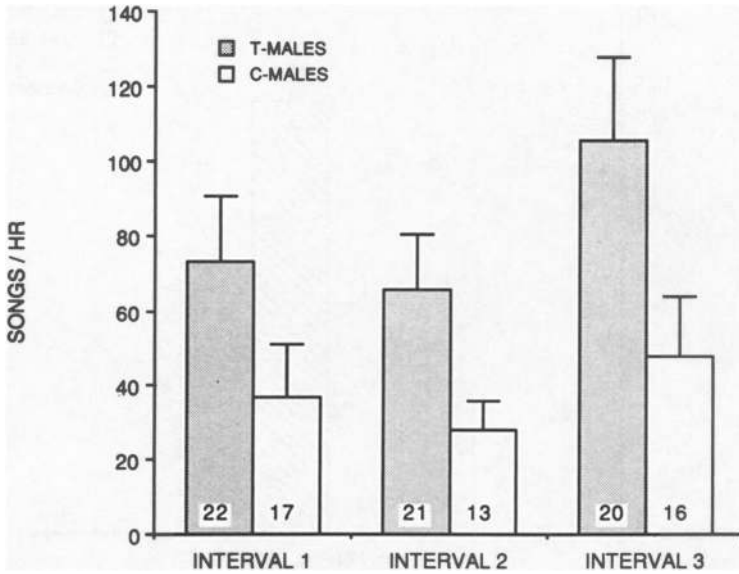


FIG. 8.—Audible song rates (mean \pm 1 SE) of T- and C-males during intervals when their nestings were 0–3, 4–7, and 8–10 d old, where 0 equals hatching day. (From Ketterson et al., in press, © The University of Chicago Press. Reprinted by permission.)

The *diamonds* in figure 7 are the maxima observed in each treatment group in each time period; note that the maxima in untreated males in early spring were quite similar to those produced in T-males.

*Effect of T on the Behavioral Phenotype: Vocal Behavior,
Parental Behavior, and Use of Space*

Most of our observations of male behavior have been made at the nest, where we have noted two changes that could affect fitness. Testosterone-implanted males sang more frequently than controls (Ketterson et al., in press) (fig. 8), and they fed their nestlings less frequently (Ketterson et al., in press) (fig. 9). This could have decreased survival of offspring, but females mated to T-males compensated for their mates' reduced effort by increasing their own rate of feeding (fig. 9). Thus, females are able to assess the level of parental effort of their mates. One might also expect that the added work would have potential consequences for the female, such as poor physical condition or lower survival or, perhaps, a greater tendency to desert an uncooperative male and to find a new mate before attempting to reproduce again (discussed later).

With the aid of radio transmitters attached to males' backs, we have recently begun to quantify male behavior away from the nest. We note a male's location every 30 min throughout the day for three or four consecutive days and map the distances separating the locations. Preliminary data suggest that T-males range more widely than controls (C. R. Chandler, E. D. Ketterson, V. Nolan, unpublished observations) (fig. 10). Laboratory studies of other bird species have shown

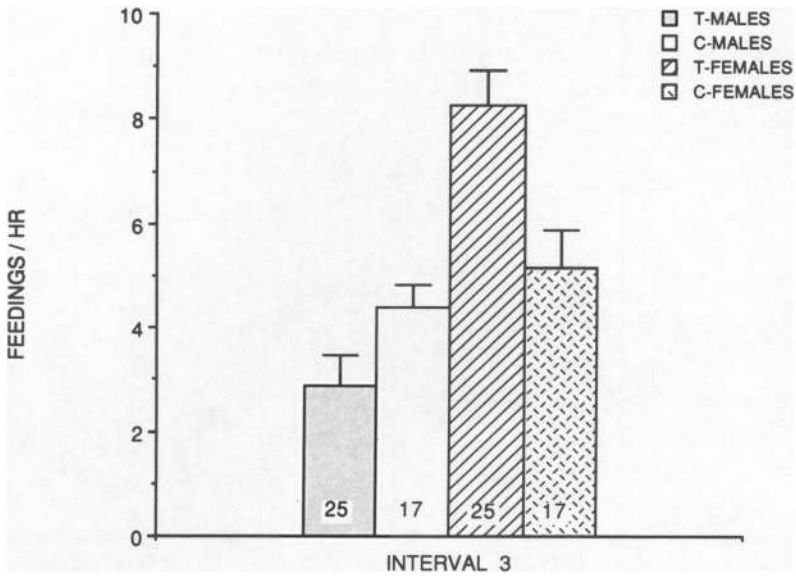


FIG. 9.—Food deliveries per hour (mean \pm 1 SE) to 8–10-day-old nestlings (interval 3 of nestling life) by male dark-eyed juncos and their mates (mean \pm 1 SE). Only the males were treated; females are categorized according to the treatment of their mates (see text). (From Ketterson et al., in press, © The University of Chicago Press. Reprinted by permission.)

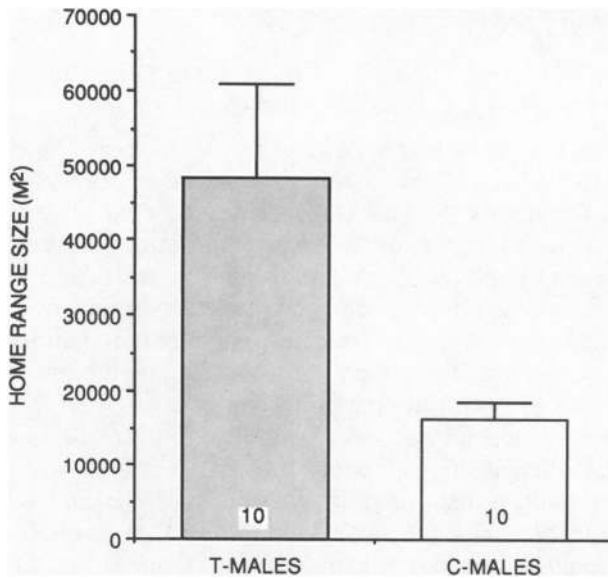


FIG. 10.—Home range size (mean \pm 1 SE) of male dark-eyed juncos, according to treatment. (From C. R. Chandler, E. D. Ketterson, and V. Nolan, unpublished observations.)

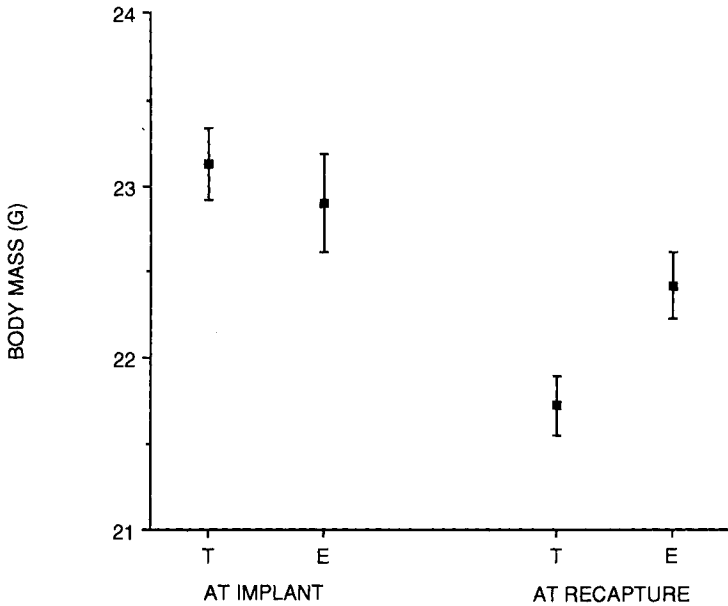


FIG. 11.—Body mass (mean \pm 1 SE) of male dark-eyed juncos at the time of implant in early spring and when recaptured, according to treatment (for both treatments, $n = 32$). (From Ketterson et al. 1991a. Reprinted by permission.)

that T increases locomotor activity (Wada 1986), which suggests that T-males may be more “restless” than controls. Restlessness might increase the likelihood that such males would encounter fertile females mated to other males (Westneat et al. 1990).

*Effect of T on the Physiological Phenotype: Body Mass,
Corticosterone, and Molt Schedule*

We have noted three physiological changes that might influence fitness of T-males (Ketterson et al. 1991a; Nolan et al. 1992). First, T-males treated in the early spring (1989) lost mass and visible subcutaneous body fat before the normal time when males lose winter fat (Ketterson et al. 1991a) (fig. 11). Later in the breeding season T- and C-males did not differ in mass (Ketterson et al. 1991a). Acceleration of loss of winter fat might increase a T-male’s vulnerability to late-spring snowstorms, which occur in the mountains and pose a risk to early breeders.

Second, plasma levels of corticosterone were higher in T-males than in controls (Ketterson et al. 1991a) (fig. 12). Whether this indicates that elevated T affects the ways in which males respond to potential stressors is not known (Siegal 1980), nor do we know whether prolonged exposure to elevated T or corticosterone might lead to immunosuppression and increased susceptibility to

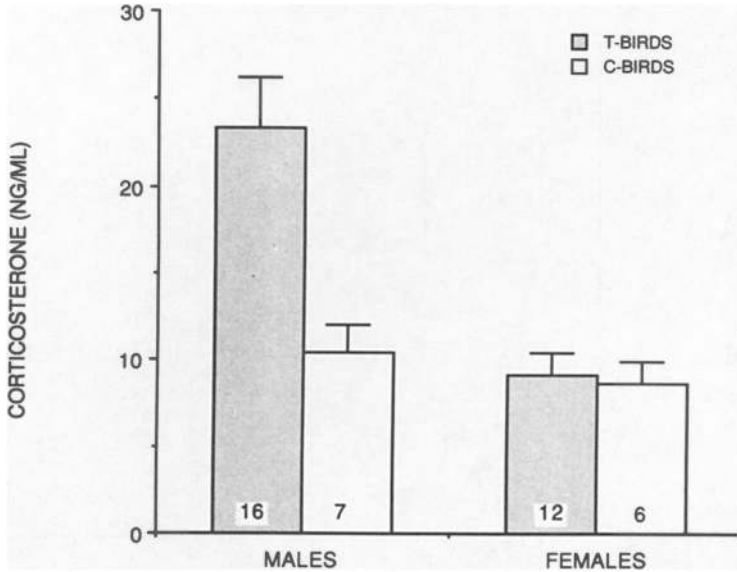


FIG. 12.—Plasma concentration of corticosterone (mean \pm 1 SE) in male and female dark-eyed juncos. Only males were treated; females are categorized according to treatment of their mates. (From Ketterson et al. 1991a. Reprinted by permission.)

disease (Chrousos et al. 1988; Zuk 1990). These questions are currently under investigation.

Finally, T delayed molt (Nolan et al. 1992) (fig. 13). Of 10 T-males still carrying implants and examined in October 1990, only six had begun to molt their primary (principal wing) feathers. All of the nine control males had renewed their primaries or were far advanced in molt. A complete molt after breeding is an annual event in juncos, but T-males whose implants we did not remove and that returned to breed in 1991 had failed to molt; their plumage was very degraded. Worn plumage almost certainly provides less effective insulation and probably impairs maneuverability in flight.

Thus, both premature and unusually prolonged exposure to high plasma levels of T have clear effects on behavior and physiology, but do these translate into differences in fitness?

Correlates of Fitness

We compared T-males to controls for various measures of fitness, including territory acquisition, mate acquisition, apparent reproductive success, extrapair fertilizations, mate retention, overwinter survival, and the mate's physical condition and overwinter survival (table 1). These correlates of fitness are arranged approximately chronologically according to the sequence of events in the breeding season and are the major components of fecundity selection and survival

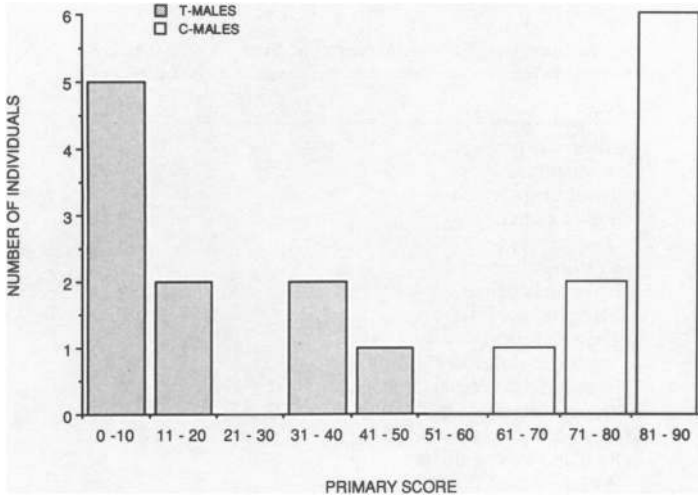


FIG. 13.—Frequency distribution of the molt score of male dark-eyed juncos, according to treatment. Juncos have nine primaries on each wing and were given a score of 10 for each right-wing primary they had replaced. Thus, individuals with a score of 90 had completed primary molt, those with a score of 0 had not begun, and those with intermediate scores were in the process of molting. (From Nolan et al. 1992. Reprinted by permission.)

selection. However, not all the measures are independent. To take an obvious example, the number of young that leave the nest cannot be greater than the number of eggs hatched.

Territory acquisition.—For males present in early spring prior to territory formation, we compared the proportion of T-males and controls that subsequently acquired territories in the study area (E. D. Ketterson and V. Nolan, unpublished observations from 1989) (fig. 14). For old males (second breeding season and beyond), in which fidelity to the former territory is nearly complete, the question was whether the hormone would affect the likelihood of reacquiring last year's territory; for young (first-year) males, the question was whether T would influence the likelihood of succeeding in the attempt to gain a territory in the area (recall that there are "floater" nonterritorial males, almost always young) (Ketterson et al. 1991*b*).

Among 35 adult T-males present in the study area before territories were established, 25 (71%) acquired territories there, as compared to 24 of 37 adult controls (65%). Among first-year males, 15 of 33 (46%) T-males acquired territories, as compared to 13 of 34 (38%) controls. Neither difference was significant; the trend slightly favored T-males in each age class.

Mate acquisition.—Based on Wingfield's (1984) observation of polygyny in male song sparrows that he implanted with T, we predicted a similar result in juncos. However, it did not occur (Ketterson et al., in press; E. D. Ketterson and V. Nolan, unpublished observations). Two T-males whose implants we did

TABLE 1
CORRELATES OF FITNESS MEASURED: SUMMARY OF TRENDS

	T-Males	C-Males
Territory acquisition	+	
Mate acquisition		=
Apparent reproductive success:		
Eggs hatched		=
Nest success		=
Nestlings fledged		+
Mass at fledging		+
Fledgling survival		?
Time to renest		+
Size of replacement clutch		+
Eggs hatched, replacement clutch		+
Extrapair fertilizations/fertility		?
Mate condition, mate retention:		
Female mass at fledging	+	
Within-season fidelity		+
Annual survival		+
Between-season fidelity	+	
Minimum overwinter survival:		
Summer removal	+*	
Autumn removal		=
No removal		+*
Total	4	8

NOTE.—The symbol + indicates treatment group with higher performance (which is the same as the treatment with the greater mean, except for renesting interval, where lower values indicate higher performance). Note that + does not indicate significant statistical differences, only trends, and the trends were often weak (see tables 2 and 3). The symbol = indicates that two means were the same. Nest success means that at least one fledgling left the nest. The symbol * indicates variables for which treatment groups differed significantly ($P < .05$; see fig. 15).

not remove returned next spring in faded and much abraded plumage, having failed to undergo prebasic molt. Despite their abnormal appearance, both obtained mates.

Apparent reproductive success.—We found no significant differences in the seven measures of apparent reproductive success listed in table 2 (Ketterson et al., in press; E. D. Ketterson and V. Nolan, unpublished observations). Success is “apparent” because we assumed that each male sired all young in his mate’s nest (but see below). Because our sample sizes are relatively small, the power of these tests is not high (power reported in Ketterson et al., in press). Still, the P values (table 2) indicate that the treatment groups were quite similar in all these measures.

To relate reproductive success to the fact that T-males fed young less frequently than C-males, the two treatments groups differed only slightly in production of fledglings per brood, presumably because the T-females compensated for

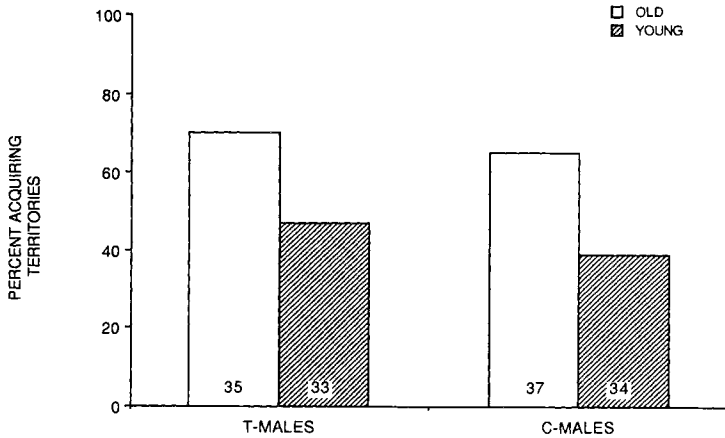


FIG. 14.—Percentage of male dark-eyed juncos that acquired territories on the study area in 1989, according to age (see fig. 7) and treatment (V. Nolan and E. Ketterson, unpublished observations).

TABLE 2
CORRELATES OF FITNESS: APPARENT REPRODUCTIVE SUCCESS

	T-PAIRS		CONTROLS		P
	\bar{X} (n)	SE	\bar{X} (n)	SE	
Eggs hatched, clutch A	3.5 (27)	.12	3.5 (18)	.22	.696
Nest success (%)	33.3 (81)	. . .	33.3 (93)999
Nestlings fledged, clutch A	3.1 (26)	.18	3.3 (15)	.25	.460
Mass at fledging (g)	16.9 (21)	.26	17.2 (15)	.28	.360
Renesting interval (d)	8.2 (16)	.95	7.1 (15)	.66	.350
Clutch size, clutch B	3.6 (14)	.13	3.8 (10)	.20	.560
Eggs hatched, clutch B	3.2 (9)	.41	3.4 (11)	.25	.870

NOTE.—Comparisons were by Mann-Whitney *U*-test (clutch size, number hatching, renesting interval), *t*-test (nestling mass, renesting interval), or χ^2 (% nesting success, i.e., proportion of nests producing at least one fledgling). Data are from Ketterson et al. (in press), except nest success (E. D. Ketterson and V. Nolan, unpublished observations); all probability values are two-tailed.

their mates' reduced effort. We note that controls outperformed experimentals in five of the seven measures.

Genetic analysis of paternity.—Reliable measures of reproductive success require determination of the paternity of young. Our collaborator P. Parker has completed a preliminary (unpublished) genetic analysis. She analyzed hypervariable DNA of the male, the female, and the offspring in 13 families. Some pairs produced more than one brood, and the total number of broods analyzed was 18. There were 13 putative fathers (six T-males and seven C-males), 11 putative mothers (two of the females had two mates in succession), and 52 offspring. She also analyzed DNA fingerprints of six males (one control and five experimental) that held territories adjoining those of one or more of these families.

TABLE 3
CORRELATES OF FITNESS: MATE CONDITION, SURVIVAL, AND RETENTION

	T-PAIRS		CONTROLS		P
	\bar{X} (n)	SE	\bar{X} (n)	SE	
Female mass, day 10 (g)	20.4 (20)	.26	20.3 (14)	.22	.680
Mate fidelity, within season (%)	95 (20)	...	100 (13)664
Female return rate, annual (%)	42 (60)	...	46 (48)424
Mate fidelity, between seasons (%)	86 (14)	...	72 (15)411

NOTE.—Comparisons were by *t*-test (female mass) and χ^2 or Fisher's exact (other variables); all probabilities are two-tailed. Data from Ketterson et al. (in press), but female return rate and mate fidelity between seasons were supplemented with E. D. Ketterson and V. Nolan, unpublished observations made during 1990.

DNA was analyzed as in Rabenold et al. (1991). In offspring that were assignable to both putative parents, the average number of unattributable bands was 0.230, and the average number of bands on which exclusion was based was 10 (extremes, 5–16). Twelve of the 52 offspring (23%) were sired by males other than the putative father, and all of these young were assignable to neighborhood males. In all cases the putative female parents were the mothers of the offspring; that is, none of the eggs had been introduced by parasitizing females (and we have no evidence that intraspecific parasitism occurs in our population).

Testosterone-implanted males sired 16 of their 20 putative offspring (80%); control males, 24 of their 32 (75%). Interestingly, all 12 offspring that were produced by extrapair fertilizations were sired by control males ($n =$ three males). None of the 11 T-males sired any young through extrapair fertilizations, whereas three of eight C-males did so (Fisher's exact $P = .107$). We cannot reconcile the tendency revealed by this preliminary genetic analysis with the behavioral data indicating that T-males move over a larger area than C-males.

Female condition, female survival, and mate retention.—We found no evidence that reduced male parental contribution and compensatory efforts by the female led to loss of physical condition by the female or to significantly more frequent desertion of the male by the female (Ketterson et al., in press) (table 3). Females paired to T-males (T-females) weighed slightly more than mates of C-males at the time their young left the nest, and they were slightly more likely to change mates within breeding seasons. They were somewhat less likely to return in the year following the experiment, but when both females and their mates returned, T-females were somewhat more likely to remate with their previous mate than were control females.

Survival of males.—Survival was affected by treatment, although in rather unexpected ways (fig. 15). For individuals whose implants we removed in summer (July, August 1987, 1988, 1989), T-males were significantly more likely than C-males to return in the year following treatment (fig. 15, panel 1; modified from Ketterson et al., in press, supplemented with E. D. Ketterson and V. Nolan, unpublished observations collected during 1990). Stated another way, males

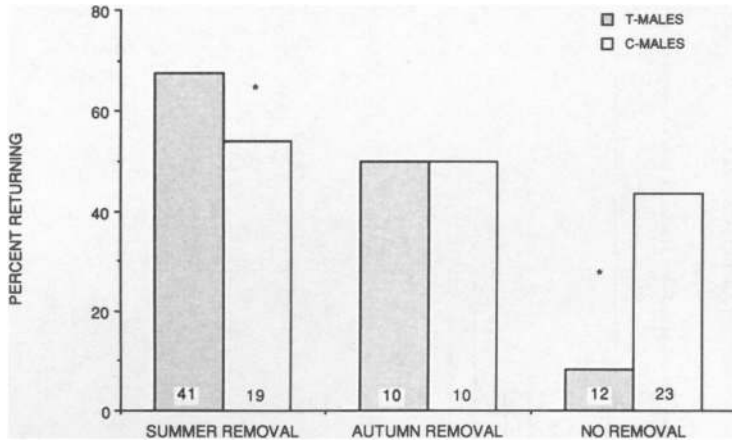


FIG. 15.—Minimum survival (% returning to the study area in the year following treatment) of male dark-eyed juncos, according to treatment and time when the implants were removed. Asterisk indicates $P < .05$, χ^2 test (based on Ketterson et al., in press; E. Ketterson and V. Nolan, unpublished observations; Nolan et al. 1992).

known to be alive in late summer were more likely to survive to the following spring if they had been treated with T in the preceding summer. We have no explanation for this result, which suggests a delayed effect of treatment.

Males whose implants we removed in autumn (October 1990) were equally likely to return the following spring, regardless of treatment (Nolan et al. 1992) (fig. 15, panel 2). Thus, even though T delayed molt by at least a month in the experimental males, they molted completely after implant removal, and the delay had no detectable effect on their survival.

Males whose implants we did not remove at all were significantly less likely to return the following spring (Nolan et al. 1992) (fig. 15, panel 3), which we interpret as the effect of greater overwinter mortality. Such mortality may have been attributable to the loss of insulation or maneuverability, but it also may have resulted from some effect of T on the tendency to join flocks in winter, migrate downslope for the winter, or accumulate winter fat reserves.

Do Juncos Vary in Any Measure of Reproductive Fitness?

Annual reproductive success.—The similarity in apparent reproductive success of T- and C-males led us to ask how much individual male juncos vary in reproductive success. Figure 16 classifies males according to number of fledglings that left the nest during single breeding seasons and according to treatment (V. Nolan and E. D. Ketterson, unpublished data from 1989 and 1990). Fifty-two percent of a total of 52 males left no offspring, 37% left one to three, and 12% left more than three. Interestingly, the individual that appeared to leave 11 fledglings was one of those analyzed by DNA fingerprinting, which revealed that five of the 11 offspring were sired by another male. Obviously, data like those in figure 16 will ultimately need to be corrected after measures of true paternity have been made.

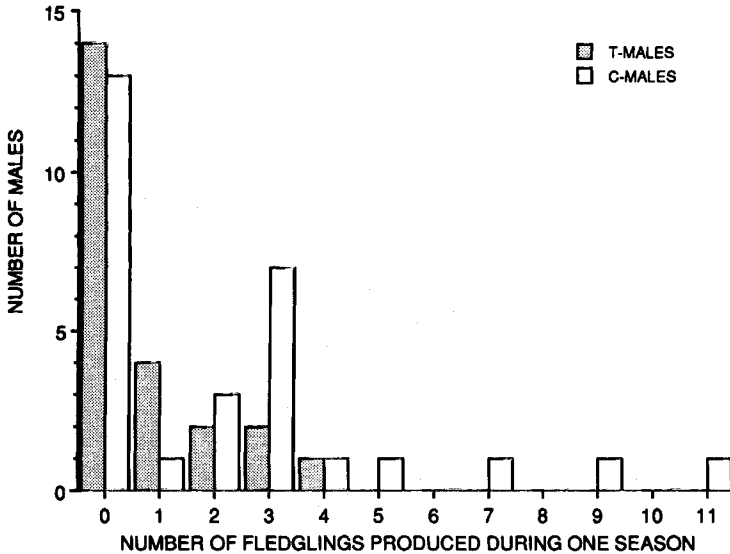


FIG. 16.—Frequency distribution of numbers of fledglings apparently produced by male dark-eyed juncos during single breeding seasons, according to treatment (data from 1989 and 1990) (V. Nolan and E. Ketterson, unpublished observations).

Nevertheless, it is clear that males vary considerably in offspring production, even though the two treatment groups did not differ significantly. This suggests to us that major determinants of reproductive success may be events beyond the control of the individual's choice of behavioral options, that is, that behavior and fitness are not tightly linked. Among Carolina juncos, nest predators (rodents, especially the chipmunk, *Tamias striatus*) play an extremely important role in determining whether a pair produces offspring, and the density of predators can vary between years by as much as an order of magnitude (E. D. Ketterson and V. Nolan, personal observations; J. Wolff, personal communication). This variation appears to be attributable to fluctuations in the annual production of acorns, a major food of rodents in our study area.

CONCLUSIONS

Treatment with T has various phenotypic consequences in male juncos: it reduces the frequency of feeding nestlings, increases song rate, accelerates loss of winter fat deposits to the low level characteristic of the breeding season, leads to elevated corticosterone, and postpones or prevents prebasic molt (Ketterson et al. 1991a, in press; Nolan et al. 1992). It is almost certainly true that, even for T-dependent traits, there is more to the regulation of their expression than simply the concentration of T in the plasma, but our study shows that alteration of the normal seasonal pattern of plasma T leads to alterations in the phenotype.

However, most of the correlates of fitness that we have investigated thus far

do not appear to be much affected by the phenotypic alterations induced by elevated T. Returning to table 1, controls weakly outperformed T-males on eight of the 16 correlates measured, T-males somewhat outperformed controls on four, and on four the treatment groups were equal. However, only two of the comparisons were statistically significant, and in all but one of the nonsignificant comparisons the means of the treatment groups differed by less than 10%. One of the significant differences was paradoxical: males with elevated T during summer were more likely to survive the following winter, after the hormone had been removed. The other significant difference was predictable from an adaptive viewpoint: males that did not molt did not survive as well over winter. Two important correlates of fitness that remain to be measured are true paternity as determined by genetic analysis and survival of offspring between nest leaving and independence.

We note as caveats, first, that the correlates we used are not true measures of fitness because they do not follow individuals from zygote to zygote but instead cross generations (i.e., the parental and the filial). Second, they are not independent. Therefore, we regard them simply as indicators of relative performance. Finally, other measures of fitness—not yet measured by us—might have been more sensitive to the behavioral and physiological changes we induced with T than the ones we monitored. Nevertheless, we return to the question of whether significant deviations from the normal seasonal pattern of plasma concentrations of T in male juncos lead to results that are paradoxical, neutral, or, as would be expected from an adaptationist perspective, disadvantageous.

In some respects, our results support an adaptive interpretation. With regard to the temporal pattern of high T, wide deviations in the onset or the termination of peak levels would probably increase mortality. Testosterone-implanted males were leaner in spring, when severe weather is still likely, and maintenance of high T into autumn evidently increases mortality. The effect of mimicking the pattern seen in polygynous species, with T elevated only throughout the normal breeding season, is less clear. To detect such effects, we look to the measures of reproductive success in years when we elevated T only during breeding: T- and C-males did not differ significantly in any measure examined so far. We might argue that the nonsignificant differences (generally favoring C-males) that we observed in reproductive success would predict larger, biologically meaningful differences under more severe environmental circumstances. If this is correct, we would conclude that in other years or at other places prolonged high T would produce measurable decreases in fitness. For example, if food were in short supply, reproductive success of T-males might be lower because their females would be unable to compensate for reduced male parental care. In turn, under such conditions females might more frequently desert males that were poor parents. In this adaptive view, spatial and temporal variation in selective regimes would be responsible for maintaining the status quo.

Alternatively, if we take our observations to mean that the treatment groups would not differ, then we may conclude that a wide range of behavioral and physiological phenotypes is effectively neutral in the junco. If this is true, then many of the important determinants of survival and reproduction in the field may

be random events that override individual variation in behavior and physiology, and fitness profiles in nature may be flatter than we have come to expect.

At this stage of investigation, we do not attempt to discriminate among these possibilities, and evolutionary biologists probably would disagree as to which is most likely to be correct. We continue to measure other potential fitness correlates of testosterone, especially the frequency of extrapair fertilizations, to monitor reproductive success in order to determine how much annual variability there is, and to seek further phenotypic consequences, both behavioral and physiological, of elevating testosterone. We also remain open to the possibility that chance is a major determinant of reproductive success and survival in the juncos that we study.

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