

Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature

ELLEN D. KETTERSON¹, VAL NOLAN, JR.¹, MICHELLE J. CAWTHORN²,
PATRICIA G. PARKER³ & CHARLES ZIEGENFUS⁴

¹ Department of Biology and Center for the Integrative Study of Animal Behavior,
Indiana University, Bloomington, IN 47405, USA

² Department of Physiology and Health Science, Ball State University, Muncie, IN 47306, USA

³ Department of Biology, The Ohio State University, Columbus, OH 43210, USA

⁴ Department of Mathematics, James Madison University, Harrisonburg, VA 22801, USA

Perhaps the best way to determine whether and how traits of organisms are currently adaptive is to alter them experimentally and compare the relative fitness of altered and unaltered individuals. We call this method phenotypic engineering. To the extent that natural selection moulds organisms on a trait-by-trait basis, we would expect fitness of unmanipulated (control) individuals to be higher than that of experimentally altered individuals. However, other outcomes are possible and of interest. If, for example, a single trait were altered and the fitness of manipulated and unmanipulated organisms were found to be similar, we might conclude that selection is not currently operating on the altered trait. Phenotypic engineering with hormones describes an experimental approach to the study of adaptive variation in suites of traits that are hormonally mediated and correlated in their expression. A likely outcome of such manipulations is that some traits would be altered so as to elevate fitness but that changes in other, correlated traits would lower fitness. If the net effect were to depress fitness, a process by which natural selection shapes and maintains organisms as integrated units would be demonstrated. We have employed this approach in studies of the Dark-eyed Junco *Junco hyemalis*, a small passerine whose reproductive success varies with the abundance of nest predators. We treated males with testosterone, documented the phenotypic consequences and related these to various measures of fitness. Summarizing results to date: Behavioural comparison of males treated with testosterone (T-males) and control males (C-males) shows that T-males sing more frequently, are less attentive to offspring, have larger home ranges and are more attractive to females. Physiologically, testosterone accelerates entry into breeding condition in spring (loss of winter lipid stores) and results in higher levels of corticosterone. If exposure to testosterone is prolonged beyond the breeding season, pre-basic moult is delayed or prevented. We are currently comparing T- and C-males with respect to corticosteroid binding proteins, sperm reserves, response to nestling vocalizations and neuroanatomy. The relationship between testosterone-induced phenotypic variation and fitness is still under study. When treatment extends well beyond the breeding season, testosterone significantly reduces survivorship; otherwise it does not. With respect to apparent reproductive success (i.e. estimates of paternity that are not based on genetic analysis), more young leave the nests of C-males than of T-males, but treatment groups do not differ in the number of young that reach independence. Preliminary data on realized reproductive success (i.e. number of genetic offspring sired) suggest that production as the result of extra-pair fertilizations is greater in T- than in C-males but that T-males lose paternity of more of the offspring of their social mates to other males. Continued investigation will, we hope, reveal the factors governing the trade-offs between male mating effort and parental effort and between survival and current reproduction, as well as the frequency with which the typical phenotype outperforms one that has been experimentally altered.

In this paper we have two objectives. The first is to describe methods and a rationale for using phenotypic manipulations to assess the extent to which naturally occurring phenotypic variation is maintained by natural selection. For this we include key examples but do not attempt a thorough review of the literature. In particular, we attempt to emphasize the potential insights to be gained through hormonal manipulations, because hormones have the property of simultaneously changing suites of correlated traits that share common physiological control mechanisms. Manipulations with hormones should, therefore, shed light on the mechanisms underlying organismal integration and certain kinds of trade-offs. The second objective is to review our own studies of the Dark-eyed Junco *Junco hyemalis* in which we have manipulated the seasonal duration of peak levels of plasma testosterone, quantified behavioural and physiological phenotypic responses to treatment (Table 1) and attempted to relate the phenotypic alterations to fitness (Ketterson *et al.* 1991b, 1992, Ketterson & Nolan 1992, 1994, Nolan *et al.* 1992, Chandler *et al.* 1994, 1995). We also refer to unpublished studies in progress by M.J. Cawthorn, C.R. Chandler, L.A. Christensen, D.A. Enstrom, A. Houtman, T.L. Kast, P.G. Parker, S.A. Raouf and C. Ziegenfus and their colleagues.

PHENOTYPIC ENGINEERING: EXPLORING THE SHAPE OF PHENOTYPIC VARIATION

Phenotypic engineering is a method for studying the adaptive significance of organismal traits (Ketterson & Nolan 1992, Ketterson *et al.* 1992). The method calls for treating organisms so as to modify some aspect(s) of their phenotype, then quantifying the effects of the treatment and comparing the performance of the modified individuals with that of controls. For example, one might pose the question, "Why are bluebirds blue?" (or why are some bluer than others?) and attempt to answer it by determining the relative fitness of bluebirds that have been rendered non-blue by some experimental manipulation.

Clearly, ornithologists have been performing this kind of

Table 1. Summary of phenotypic responses in Dark-eyed Juncos to treatment with testosterone

Behaviour	Phenotypic effects	
	Physiology	Morphology ^a
Song	Corticosterone	Brain nuclei
Feeding nestlings	Body mass	Syrinx
Home range size	Molt schedule	Gonads
Attractiveness	Sperm reserves	Sperm morphology
Nest defence	Corticosterone-binding protein	

^a Still under study.

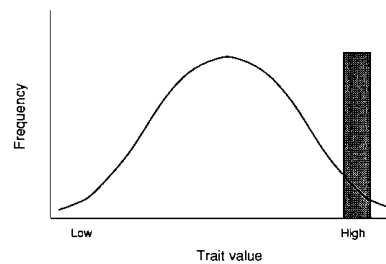


Figure 1. Common frequency distribution of trait values: typically, metric biological traits are normally distributed. The histogram bar at the right shows that by manufacturing individuals with extreme trait values it becomes feasible to make large-scale comparisons of typical and atypical individuals.

manipulation for decades without giving it a formal name (e.g. Peek 1972, Smith 1972, Andersson 1982), nor are concepts of 'engineering' new. Note the familiarity of terms such as 'genetic engineering' and the more recent 'allometric engineering' (Sinervo & Huey 1990). Nevertheless, we suggest that naming the method and articulating how various outcomes might be interpreted may help to consolidate efforts to probe the evolutionary significance of phenotypic variation, as well as the extent to which variation is shaped by selection on a trait-by-trait basis (Travis 1989).

Most quantitative (continuously varying) biological traits, such as tail length, brightness of plumage and age at onset of thermoregulation, are known, or presumed, to follow a normal distribution. The majority of individuals exhibit values close to the population mean, and relatively few express values near the extremes (Fig. 1). If we attempt to determine how such distributions are maintained by natural selection and seek to relate trait values to fitness, we face a quandary. How are we to make meaningful comparisons when there are so few individuals with extreme values? However, if we *manufacture* large numbers of individuals with extreme trait values (Fig. 1), we can investigate experimentally whether their fitness is higher or lower than that of the most abundant phenotype.

In fact, such manipulations can be used to ask whether phenotypes not normally found in nature could survive and compete. To visualize the effect of such manipulations, imagine the histogram bar in Figure 1 to be even farther to the right or to the far left. As examples, investigators interested in female choice have used environmental manipulations to produce individual songbirds that sing abnormal songs (Williams *et al.* 1993, Freeberg *et al.* in press) and physiological manipulations to produce individuals that cannot sing at all (McDonald 1989). Similarly, other investigators have artificially elongated the tails of birds, sometimes producing tails considerably longer than any occurring naturally (Andersson 1982).

Three possible relationships between trait values and fitness are depicted in Figure 2 (Falconer 1989, Stearns 1992, see also Ketterson & Nolan 1992). Investigators with an adaptationist perspective, expecting that most distributions

of existing phenotypes are maintained by normalizing natural selection, might predict that selection would act against animals that deviate from the norm (see curve 1). Another possible result, one in which the extreme form outperforms the norm (depicted in curve 2), has been termed paradoxical (Ketterson & Nolan 1992) because it leads us to ask why the extreme, fitter phenotype is not more common in nature; that is, why directional selection has not led to an increase in its relative frequency. Also possible is the result depicted in curve 3, the flat curve that suggests little or no relation between trait value and fitness, at least over a wide range of trait values. This 'neutral' result would obtain if selection were neither normalizing nor directional.

Plumage manipulations of the Red-winged Blackbird *Agelaius phoeniceus* provide a classic example of phenotypic engineering with an outcome that points to the adaptiveness of an existing trait. Many populations of Red-winged Blackbirds breed colonially, and males, when displaying to conspecific males or females, spread their wings and erect the feathers of their bright red-coloured epaulets (primary coverts). Peek (1972) and Smith (1972) independently conducted experiments in which they captured mated males, blackened their epaulets with dye and released the birds onto their territories. Most of the males lost their territories (i.e. disappeared), but those that retained them also tended to retain their mates. Subsequent investigators have measured the effect of devocalization (muting), of clipped epaulets, and of coloured leg rings on the ability of Red-winged Blackbirds to maintain their territories and on their attractiveness to females (Smith 1976, Røskaft & Rohwer 1987, Metz & Weatherhead 1991, 1992); these studies have also shown that individuals that deviate from the norm are selected against.

But individuals that depart from the norm are not always selected against, as a second example shows. N. Burley (unpubl.) recently completed a study of social preferences of male and female Long-tailed Finches *Poephila acuticauda* and Zebra Finches *Taeniopygia guttata*. These two Australian species belong to the family Estrildidae (or the subfamily Estrildinae within the Passeridae), and none of the more than 100 members of this group are crested, nor are any close relatives. Yet when Burley glued feathers to form a crest on the heads of males and gave females a choice of associating either with uncrested control males or with males having crests of different colours (red, black or white), the females of both species showed a marked preference for males with white crests. Similarly, male Long-tailed Finches preferred conspecific females with white crests over controls with no crests and also over females with crests of other colours. Only male Zebra Finches preferred normal, uncrested females. It would seem that selection would favour crested male finches, but they do not exist.

Much of this paper deals with an example of the third type of curve (Fig. 2, curve 3): manipulations that markedly change the phenotype but have very few detectable effects on fitness. Hence we will postpone discussion of this alternative, except to anticipate what it might mean when in-

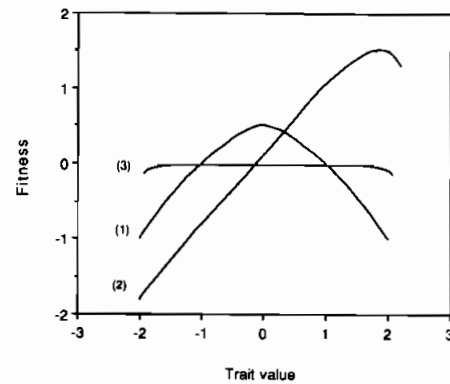


Figure 2. Hypothetical fitness profiles relating trait values to fitness. Curve 1 depicts an adaptive outcome because fitness is highest among typical individuals; curve 2 is linear and rising and depicts a paradoxical relationship in which individuals that deviate widely from the norm have greater fitness; curve 3 is flat over most of the range of trait values and depicts a neutral relationship in which fitness is relatively independent of trait value. Adapted from Falconer (1989), Ketterson and Nolan (1992), Stearns (1992).

vestigators manipulate traits and fail to demonstrate normalizing selection in action. First, if selection differentials are small, such that the fitness of altered individuals is only slightly lower than that of normal individuals, it may not be possible to obtain the enormous samples required to show these small differences in fitness. This is a problem of statistical power (Cohen 1988). Second, investigators may fail or be unable to measure all the relevant components of fitness that are affected by the manipulation. Clearly, to grasp the role of selection in shaping phenotypic variation, it is essential to track all the components of fitness affected by any particular trait, but this is not always possible.

But suppose the failure to show that typical individuals have higher fitness than modified individuals reflects the true situation—during the experiment there was no difference in fitness between typical and experimentally altered individuals. What could that mean? The usual explanation would be that the study was performed at a time or place in which selection was not operating. It is important to report such 'negative' results if we are to assess accurately the degree to which selection is episodic in nature. Another possible explanation pertains to correlated traits. If the trait under consideration is linked in its expression to other traits and those traits do affect fitness, then it may be selection on those other traits that maintains the *status quo*. This kind of finding it also important because it enhances our understanding of natural selection and organismal integration.

Still another possibility may be termed compensation, and this possibility has received the least attention. If the organism under study is phenotypically resilient and has the ability to compensate for the disadvantageous manipulation of one trait by modifying the values of others, then experiments on the focal trait may not alter fitness. As a hypothetical example, if males were rendered less colourful and thus

initially less attractive to females, they might respond by singing more frequently, overcoming the disadvantage so that no net change in fitness results. This alternative may be most likely to apply when investigators manipulate physiological and behavioural traits, as opposed to morphological ones. What appears to a physiologist as homeostasis, or to a behavioural biologist as reallocation of effort, might appear to an evolutionary biologist as a flat fitness profile. Again, for a complete understanding of natural selection and trait evolution, it is important to document cases of compensation or phenotypic resiliency.

Phenotypic engineering with hormones

Thus far our emphasis has been on manipulations of single traits, and we now consider how hormones may be used deliberately to manipulate several traits simultaneously. Hormones, as compounds that affect the action of receptive tissues throughout the organism, are an important interface between the environment and the genome. By responding to activating signals from both genes and environment and transducing these signals via gene transcription or neuronal transmission, hormones exert considerable influence on the phenotype (e.g. Epple & Stetson 1980, Knobil & Neill 1988, Wada *et al.* 1990, see also Schumacher 1990). Seen from this perspective, hormones provide an important mechanistic link between genes and life history traits. Further, because hormonal manipulations can simultaneously modify behavioural, morphological and physiological traits, the use of hormones to engineer phenotypes provides a powerful tool for study of how interactions among traits contribute to phenotypic variation and influence fitness.

This is particularly true if the traits under consideration are components of a trade-off. Trade-offs can be defined in various ways. One is the situation that exists when a series of traits has a common cause, and the beneficial effects of changes in some traits are cancelled out by the accompanying detrimental effects of changes in others. Trade-offs result in a loss in 'potential fitness' because the common cause prevents the organism from attaining a benefit without experiencing a loss (Williams 1957, Stearns 1989, 1992, Roff 1992). Trade-offs are usually discussed in terms of pleiotropy, but they can also be readily discussed in terms of their physiological basis, particularly if that basis is hormonal (Endler 1995). Suppose, for example, that elevated hormone secretion were to increase the frequency of sexual displays in male birds, but because the hormone also has other tissues as targets, it also increases susceptibility to disease (Zuk 1990, 1994). If such males were more attractive to females but suffered higher mortality because of disease, these interlocking hormone-dependent traits would be both beneficial and costly and the hormone would be the proximate cause of the trade-off that resulted.

Several workers have urged that greater effort be devoted toward uncovering the physiological mechanisms that underlie trade-offs, particularly those associated with the endocrine system (Stearns 1989, West-Eberhard 1989, Ligon

et al. 1990, Zuk *et al.* 1990, Moore 1991, Zuk 1994), and recent studies that have employed hormones in an experimental approach to life history evolution have proven fruitful (Fox 1983, Wingfield 1984, Marler & Moore 1988a,b, 1989, 1991, Silverin 1991, Sinervo & Licht 1991a,b, Ketterson & Nolan 1992, Ketterson *et al.* 1992, Glickman *et al.* 1993, Sinervo 1993).

The endocrine approach would seem to be particularly useful when employed to investigate trade-offs that affect male fecundity, i.e. the relative importance of mating effort v parental effort. A male may achieve greater fecundity by increasing the number of females he fertilizes and also by increasing the level of his paternal care (and thus the likelihood that his young will survive) (Maynard Smith 1977, Clutton-Brock 1991); but the two kinds of effort compete for time and energy and are widely believed to be negatively correlated, so that selection cannot simultaneously act positively on both mating effort and parental effort in the same individual. A single hormone, testosterone, appears to play a pivotal role in regulating the balance of mating effort and parental effort (Balthazart 1983, Wingfield *et al.* 1987, 1990, Ball 1991, Balthazart & Ball 1993) and has been the subject of much study.

Testosterone in birds

Testosterone is intimately associated with avian reproduction (Wingfield & Moore 1988). It mediates copulatory behaviour (summarized in Balthazart & Ball 1993), promotes sperm production (Lake 1981, Scanes 1986), enhances courtship vocalizations (Wada 1981, 1982, 1986, Arnold 1982, Gyger *et al.* 1988, Harding *et al.* 1988, Nowicki & Ball 1989, Ketterson *et al.* 1992) and increases aggressiveness (Balthazart 1983, Wingfield *et al.* 1987, 1990, Archawararon & Wiley 1988, Beletsky *et al.* 1990). It also suppresses male incubation and feeding of nestlings (Silverin 1980, Hegner & Wingfield 1987, Oring *et al.* 1989, Ketterson *et al.* 1992). Furthermore, as indicated by its role in many physiological responses, testosterone can influence survival (Duffy 1989). For example, it increases locomotor activity (Wada 1982, 1986, Massa & Bottoni 1987), it influences metabolic rate (Hannsler & Prinzinger 1979, Feuerbacher & Prinzinger 1981) and it suppresses lipid storage (Wingfield 1984, Ketterson *et al.* 1991b) and the onset of postnuptial moult (Runfeldt & Wingfield 1985, Schleussner *et al.* 1985, Nolan *et al.* 1992). Testosterone has also been implicated in corticosterone metabolism (Wingfield *et al.* 1982a, Ketterson *et al.* 1991b, Wingfield 1994) and in suppression of the immune system (Zuk 1990, 1994, Folstad & Karter 1992, but see Weatherhead *et al.* 1993).

Testosterone and avian mating systems

Wingfield and his associates' pioneering studies of testosterone and avian mating systems (1987, 1990) provide comparative evidence demonstrating a link between testosterone and interspecific variation in allocation of effort between

mating and parental behaviour. Males of monogamous species (as traditionally defined, without reference to genetically determined paternity) typically provide parental care, whereas polygynous males contribute less, if any (Emlen & Oring 1977, Oring 1982, Vehrencamp & Bradbury 1984). When seasonal profiles of plasma testosterone of polygynous and monogamous species are compared, males of polygynous species exhibit sustained secretion of testosterone at peak levels for much of the breeding season, whereas monogamous males exhibit a single early peak (Wingfield *et al.* 1987). The ratio of summer to winter levels of testosterone also correlates with mating system and parental behaviour: the greater the seasonal change in testosterone, the more likely a species is to be polygynous and the less likely it is to provide parental care (Wingfield *et al.* 1990). On the other hand, in monogamous species, with their normally lower levels of testosterone, an aggressive challenge (e.g. by a territorial intruder) causes testosterone to increase more than it does in polygynous species (Wingfield *et al.* 1990). Together, this evidence suggests that polygynous males operate for most of the breeding season close to their physiological maximum of testosterone, whereas monogamous species tend to secrete high testosterone only "on demand". Our study species, the Dark-eyed Junco, is occasionally polygynous (<10% of cases), but in general it conforms to the monogamous pattern described by Wingfield *et al.* (1990, compare Ketterson & Nolan 1992).

Because patterns of secretion of testosterone correlate so closely with variability in avian mating systems, it is obviously of interest to know whether changes in testosterone metabolism are the cause of evolutionary conversions from one pattern of male mating and parental effort to another. Intraspecifically, genetic variation in testosterone-mediated traits has been demonstrated in a number of ways. Aggressiveness, for example, has been shown to be highly heritable (e.g. Boag 1982, Moss *et al.* 1982, Maxson *et al.* 1983), and selection on one of a suite of testosterone-dependent traits can lead to correlated responses in other such traits. Thus, in Japanese Quail *Coturnix japonica*, selection on mating frequency led to higher aggressiveness and increased size of the cloacal gland (Sefton & Siegel 1975, Cunningham & Siegel 1978). This finding demonstrates not only correlated responses to selection but, more importantly, the interlocking of behavioural and morphological responses to selection. Looking next for phylogenetic lability, systematic studies of the evolution of male parental care in birds have shown that care can take many forms and that some forms of care have evolved and been lost in a variety of lineages (Silver *et al.* 1985, McKittrick 1992, Ketterson & Nolan 1994). Thus, within families or subfamilies (e.g. Icterinae), males belonging to different genera may differ widely in the level of care provided.

When we combine the observations of Wingfield *et al.*'s (1987, 1990) link between profiles of testosterone and mating systems, within-species genetic variability for testosterone-related traits (heritability and correlated responses to selection) and across-species lability in patterns of care, it

seems likely to us that the physiological mechanisms underlying fecundity trade-offs are highly conserved but also that they are sufficiently flexible to allow shaping by environmental changes and natural selection (Ketterson & Nolan 1994). Thus we might expect that, at the levels of species, populations and individuals, male birds are likely—within limits and on differing time scales—to be able to match their physiologies to their environments to achieve an advantageous balance of mating and parental effort. This is a proposition that calls for experimental testing.

Experimental manipulations of testosterone

Studies in which plasma testosterone has been experimentally increased during the breeding season have demonstrated that exposure of males to testosterone during the phase when they have dependent young shifts their behaviour away from parental activities and towards other forms of reproductive effort, e.g. sexual/aggressive behaviour (Silverin 1980, Watson & Parr 1981, Wingfield 1984, Hegner & Wingfield 1987, Oring *et al.* 1989, Ketterson & Nolan 1992, Ketterson *et al.* 1992). With the exception of our own work, these studies have also shown a decrease in the number or quality of offspring produced when individual males invest less in parental care than is typical of their species.

Manipulative studies have also shown that treatment with testosterone can increase the number of mates acquired by males of some ordinarily monogamous species (Watson & Parr 1981, Wingfield 1984), and Hegner and Wingfield (1987) have speculated that testosterone might increase male fecundity by facilitating extra-pair fertilizations.

Our effort has been to identify the full array of phenotypic consequences of altered testosterone levels in males of a single species and also to determine the magnitude of any fitness costs and benefits of redirecting effort from parental care to mating. We present our methods, results and much of our discussion in modules. First we review phenotypic effects we have documented (Table 1), considering behaviour, then physiology. We then summarize findings during 2–8 years of investigation of fitness measures (depending on the measure), including reproductive success, extra-pair paternity, annual survival and mate fidelity. We believe that our study is the first to attempt to document the net effect of treatment on *both* mating and parental behaviour and on such a wide array of fitness consequences of altered investment in mating and parenting. Despite prolonged efforts, we are left with a number of unresolved questions and will conclude by describing these and our plans for future research.

MANIPULATING TESTOSTERONE IN FREE-LIVING DARK-EYED JUNCOS

Bird, study area and basic methods

The Dark-eyed Junco is an abundant, socially monogamous, usually ground-nesting passerine bird. Males are territorial

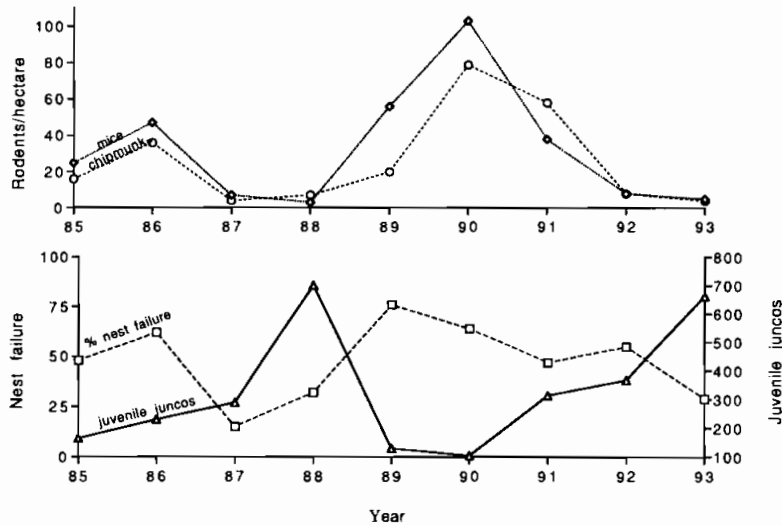


Figure 3. Rodent density and Dark-eyed Junco reproductive success at Mountain Lake Biological Station, Giles County, Virginia, 1985–1993. Rodent data from J. Wolff (unpubl.), junco data from C. Ziegenfus *et al.* (unpubl.).

during the breeding season. Females build the nest and incubate, and males and females help rear nestlings and fledglings (e.g. Wolf *et al.* 1988, 1990, 1991). Modal clutch size is four. Juncos are widely distributed in North America and, since 1983, we have studied them in the Appalachian Mountains at the University of Virginia's Mountain Lake Biological Station in Giles County, Virginia, where the species breeds at the highest altitudes (above 1000 m). Juncos raise two and sometimes three broods per season if their nests escape predators; if they do not escape, pairs renest repeatedly. In winter, they form flocks and some, but not all, individuals make short movements to lower elevations; males older than 1 year are least likely to leave the breeding altitudes (Ketterson *et al.* 1991a).

Our field season typically runs from April to August, and each year we implant 80–110 colour-ringed males. Half of these receive two 10-mm silastic implants packed with testosterone, and the other half receive empty implants (see Ketterson *et al.* 1992). Plasma levels of testosterone induced by the implants are similar to the early-season natural maximum of unmanipulated adult males, and implants cause levels to remain elevated for the entire breeding season (Ketterson *et al.* 1991b, Ketterson & Nolan 1992; radioimmunoassays using the methods of Wingfield & Farner 1975, Wingfield *et al.* 1982b, Ketterson *et al.* 1991b).

Annually, 50–60 of the implanted males settle to breed on our study area. From them, their mates and their offspring (all also colour-ringed), we collect blood samples for DNA, and from most adults we collect plasma for hormones and hormone-binding proteins. We also monitor clutch size, nest success, production of fledglings and independent young, mate fidelity during single seasons and across breeding seasons and return rates (minimum overwinter survival) of

males, females and juveniles (Ketterson & Nolan 1992, Ketterson *et al.* 1992, C. Ziegenfus *et al.* unpubl.). Our evidence indicates that all or nearly all surviving adult males, most surviving adult females and many surviving juveniles return the next year (see below).

Methods more particularly related to specific results are described in the sections in which we present those results.

Nest predation a key demographic factor in the population biology of Dark-eyed Juncos

A key feature in the population biology of these juncos is nest predation, and losses of eggs and young are attributable primarily to small mammals. Predation varies greatly across years, and the variation can be related to fluctuations in the size of small mammal populations (C. Ziegenfus *et al.* unpubl.); these fluctuations are related in turn to annual variation in mast production (Wolff in press). Since 1979, J. Wolff has carefully monitored the abundance of the nocturnal White-footed Mouse *Peromyscus leucopus* on our study area, and since 1984, he has monitored less thoroughly the abundance of the diurnal Eastern Chipmunk *Tamias striatus*. We began to measure success and failure (proportions of nests producing at least one fledgling or lost to predators, respectively) in 1983 and to capture independent juveniles produced on the study area and its environs in late summer 1985. As shown in Figure 3, mouse density, which correlates with chipmunk density, is also significantly and positively related to nest failure ($r_s = 0.85$, $P < 0.05$) and inversely related to the numbers of juveniles caught in late summer ($r_s = -0.88$, $P < 0.05$).

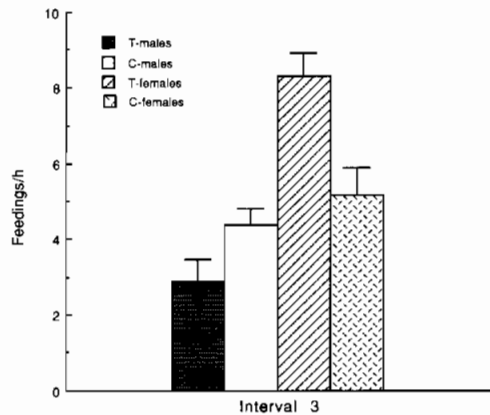


Figure 4. Testosterone and parental behaviour (nestling feedings per hour during the last third of nestling life, referred to as Interval 3, according to sex and treatment; after Ketterson & Nolan 1992). Females are categorized according to the treatment of their mates.

Effect of treatment with testosterone on behaviour

Behaviour at the nest: feeding of young, singing, nest defence

Our first insights into the effect of testosterone treatment on the behaviour of male juncos were gained by observing adult behaviour at the nest (Ketterson *et al.* 1992). As had been reported for the Pied Flycatchers *Ficedula hypoleuca* (Silverin 1980) and House Sparrow *Passer domesticus* (Hegner & Wingfield 1987), we found that T-males fed broods less frequently than C-males. We also found that the mates of T-males compensated for this decreased rate of feeding by males (compare Nolan 1978, Wolf *et al.* 1990), so that broods were fed equally often, regardless of the treatment status of their fathers (Fig. 4). Lower feeding rates by testosterone-treated males were accompanied by higher song rates than those of controls (Ketterson *et al.* 1992; Fig. 5). Together, these observations suggested that testosterone fosters behaviour associated with mate attraction and territory defence and that it does so at the expense of behaviour ordinarily directed toward offspring.

However, beyond these basic observations, we still know very little about how testosterone disrupts male parental behaviour or what leads to the compensatory reaction by females. For example, T-males go to the nest not only less often but, we suspect, also less predictably (C.R. Chandler *et al.*, unpubl.), and we would like to know more about the hormone's effect on how males allocate time to competing activities. We also do not know whether the hormone affects the size or kinds of food items brought to the nest or the total mass of the load delivered. Finally, we do not know how our treatment of the male affects his responsiveness to the young or how decreased care by the male indirectly affects the behaviour or appearance of the young so as to

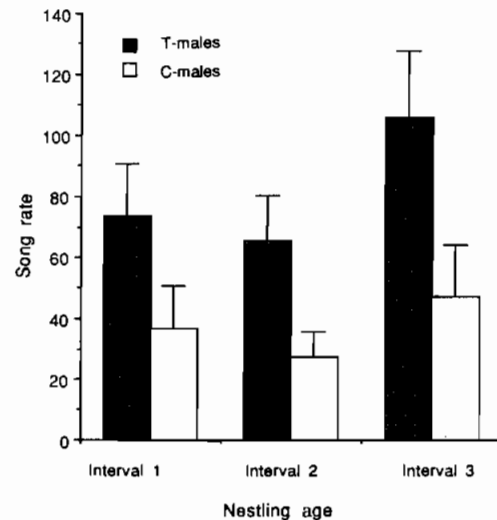


Figure 5. Testosterone and vocal behaviour (songs per hour, according to age of nestlings and treatment; after Ketterson *et al.* 1992). The nestling period, about 12 days, is divided into three approximately equal intervals.

accelerate the rate of feeding by the female. These are questions we hope to pursue.

Beyond feeding of the young, another parental role played by male juncos is nest defence. To test whether treatment with testosterone might increase or decrease the effectiveness at nest defence, M.J. Cawthorn and D. Morris (unpubl.) measured the latency and intensity of response of juncos (15 T-pairs and 18 C-pairs) to a stuffed chipmunk, a major nest predator (see above). The chipmunk was first concealed near nests that contained nestlings. Trials lasted for 10 min and began when the chipmunk was revealed by remote control. Observers noted the time elapsed before a junco detected the chipmunk (latency) and the intensity of nest

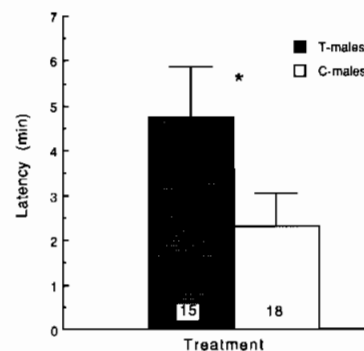


Figure 6. Testosterone and nest defence. Mean time required by male Dark-eyed Juncos to respond to a stuffed nest predator placed near their nests containing nestlings. M. Cawthorn *et al.* (unpubl.).

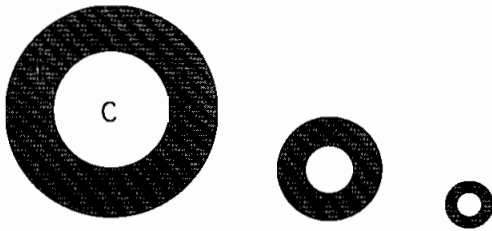


Figure 7. Testosterone and spatial activity. Depicted diagrammatically are the relative areas of three measures of use of space. Left: home range size (minimum convex polygon); centre: area in which 95% of sightings occurred; right: area in which 50% of sightings occurred. Note: larger shaded circles represent areas of T-males; inner circles represent areas of C-males. After Chandler *et al.* (1994).

defence (swoops, hits, etc.). Males that did not appear during the trial were assigned the maximum latency value of 10 min.

We found that significantly fewer T-males came to the nest and that the average latency for T-males was significantly greater than for C-males (4.7 min *v* 2.3 min, $P < 0.05$; Fig. 6). When present, T- and C-males were equally vigorous in defence, striking the model 2–3 times per minute. Interestingly, defence by females, whatever their mate's treatment group, was more intense than that by males (M.J. Cawthorn *et al.*, unpubl.).

Behaviour away from the nest: home range size and singing

To determine how testosterone affects behaviour away from the nest, we employed radio transmitters to track the movements of males. Several workers have reported hyperactivity in captive birds treated with testosterone (Wada 1982, 1986, Massa & Bottoni 1987), so it seemed likely that one response to treatment of free-living males might be an increase in home range or territory size. During 1990 and 1991, Chandler *et al.* (1994, 1995) tracked 20 juncos (ten T-males and ten C-males, half with females incubating eggs and half with nestlings), determining their location every half-hour for periods of 3–5 days.

By all measures employed, we found that the home ranges of T-males were considerably larger than those of C-males (Fig. 7). For example, during incubation the home ranges of T-males, measured as minimum convex polygons, were more than three times larger than those of C-males (mean for T-males, 6.36 ha; mean for C-males, 1.96 ha; Chandler *et al.* 1994). Although we did not measure defended area, it seems very probable that T-males also had larger territories. This is because during incubation core areas within the home range, i.e. the much smaller areas that encompassed 95% and 50% of the males' observed activities, averaged 1.51 ha (95%) and 0.31 ha (50%) for T-males, as compared with 0.33 ha (95%) and 0.09 ha (50%) for C-males

(Fig. 7). When parents were feeding nestlings, core areas were even smaller, but they were again much larger on average for T-males than for C-males (0.21 ha *v* 0.04 ha, respectively, for the 50% core areas). In general, T-males were found farther from their nests than were C-males (Chandler *et al.* 1994).

Chandler *et al.* (1994) also noted the behaviour of the radio-tracked males and found that T-males were singing on 23% of the occasions on which they were observed, whereas C-males were singing on only 6% ($P < 0.003$). Males also differed in the time spent at the nest (4% *v* 12%, $P < 0.02$), but there was no significant difference in time spent foraging (T-males 29%, C-males 38%, n.s.).

We still know very little of the ranging patterns of T- and C-males at the time their social mates are fertile (compare Saino & Møller 1995). Preliminary data (C.R. Chandler *et al.*, unpubl.) suggest that during nest-building, when females can be presumed fertile, both classes of males spend most of their time with their mates (approximately 85% of observations). However, during nest-building, J. Breeden and A. Houtman (unpubl.) placed a caged male on the territories of T- and C-males and broadcast tape-recorded song, often eliciting aggressive responses by the males whose territories were thus encroached upon. Both T- and C-males approached the intruder, but C-males were significantly more likely to be accompanied by their mates. This may suggest that, when challenged by an intruder, T-males are more ready to leave their mates unguarded. Clearly, more data are needed on the movements of males at the time their mates are fertile.

Behaviour of captive males: female mate choice

The observation that male juncos treated with testosterone sing more frequently than controls raised the question of their relative attractiveness to females. If females base their choice of mates on attributes that predict high parental effort (Burley 1986), and if song rate is inversely related to parental effort (either of which may or may not be true), we might expect females to prefer C-males. But if females rely on possible correlates of readiness to copulate or greater effectiveness in male–male competition, they might prefer T-males.

D.A. Enstrom *et al.* (unpubl.) simultaneously presented two males matched for age, one from each treatment group, to a captive female junco and asked whether females preferred to associate with T-males or C-males. The female had 30 min to assess the T- and C-males and was then given 40 min in which to express a preference. Preference was based on amount of time spent in close proximity to one male or the other. Females spent significantly more of their time with T-males ($n = 27$, $P < 0.05$; Fig. 8). Enstrom repeated the experiment after treating half of the females with estrogen, which caused them to give more pre-copulatory solicitation displays, and they again spent more time with T-males and also displayed more often to T-males ($P < 0.05$).

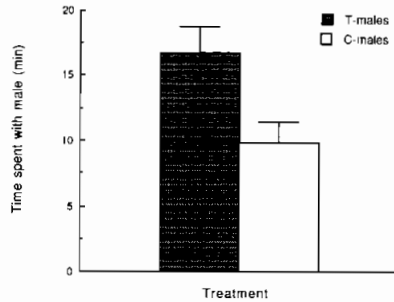


Figure 8. Testosterone and mate choice. Bars represent mean time (\pm s.e.) spent by 27 captive female Dark-eyed Juncos in active choice (perched within 0.5 m) of one or the other of a set of two simultaneously presented male juncos, one a T-male and one a C-male. Females spent significantly more time near T-males ($P < 0.01$). D.A. Enstrom *et al.* (unpubl.).

Because adult juncos with breeding experience have higher circulating levels of testosterone than do yearlings (Ketterson & Nolan 1992 and unpubl. data), Enstrom extended his mate choice trials, like those just described, and asked whether females prefer untreated adults to untreated yearlings. He found that they preferred adults (D.A. Enstrom *et al.*, unpubl.). He is currently asking whether the preference is based on hormone-dependent behaviour, such as courtship displays, or on less labile age-related traits, such as plumage colouration (Owens & Short 1995).

Potential fitness implications of testosterone-induced behavioural changes

The potential fitness implications of enhanced singing, depressed parental behaviour, diminished ability to detect potential nest predators, greater home range size (and possibly larger territories) and greater attractiveness to females are obviously numerous and great. Further, because some of these effects are probably beneficial and others detrimental, the net effect on fitness is not readily predictable.

Song has long been believed to attract females and warn off males (e.g. Kroodsma *et al.* 1982). If T-males defend more resources, as suggested by their larger core areas, their mates might be able to provision young more easily and therefore raise young equal in quality to those of control pairs, despite the lower feeding rates of the males (Chandler *et al.* 1994) (see Fig. 9). Wandering off territory probably increases opportunities to court the mates of neighbouring males and possibly to achieve greater fitness through extra-pair fertilizations. On the other hand, if T-males wander when their social mates are fertile, they may be less effective at assuring their own paternity. Further, observation has shown that male juncos can be effective nest defenders (V. Nolan & E.D. Ketterson, unpubl.), and because T-males spend less time near the nest, they are less likely to help in this capacity (Chandler *et al.* 1994, M.J. Cawthorn *et al.*, unpubl.). Finally, it is not clear whether the finding that T-males are more attractive (D.A. Enstrom *et al.*, unpubl.) should be taken to

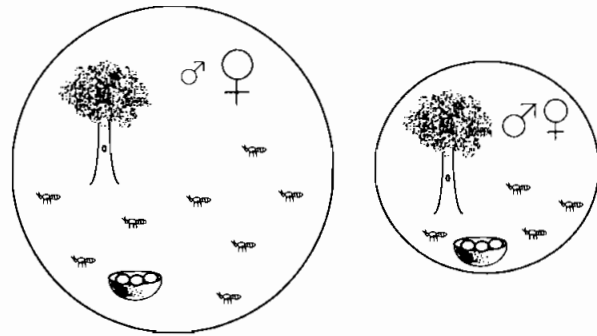


Figure 9. A hypothetical compensatory response to experimentally elevated testosterone that might result in a flat fitness profile. The left-hand figure pertains to a hypothetical male with elevated testosterone; this male contributes less parental care than his mate but defends a larger territory, which provides more food for nestlings. The right-hand figure pertains to a hypothetical male with normal testosterone levels; this male defends a smaller territory with fewer resources, but his role in offspring care is as great as the female's. The net effect is that both situations lead to the production of the same number of offspring.

mean that females would prefer males with elevated testosterone as social mates or as extra-pair copulatory partners or both. But the finding does indicate, if all other things were equal, that in nature it should be advantageous for males to elevate their testosterone levels.

In summary, certain consequences of treatment with testosterone are probably beneficial, but others seem likely to be detrimental (Fig. 9). Whether the *net effect* of testosterone-induced behavioural changes is beneficial would seem to depend on circumstances that we know to vary from time to time both within and across seasons. These include abundance of nest predators, degree of breeding synchrony among females on neighbouring territories (which depends largely on whether nest predators force females to build and lay in replacement nests, during which time they are fertile) and availability of food for young. In years of low nest predation, alternative reproductive opportunities for males would be few, as most females would simultaneously be engaged in care of broods; helping their social mates care for young should enhance male reproductive success more than would seeking extra-pair fertilizations, especially if food were in short supply. In such years, typical males (i.e. the equivalents of our C-males) that allocate greater effort to parental behaviour should be favoured. But in years when nest predation is high, causing frequent and asynchronous re-nesting, then from the male's perspective, opportunities for extra-pair copulations with fertile females would be regularly available throughout the season. In such years, especially if food is abundant and male care is not critical for much of the nesting cycle, as is often true in the junco (Wolf *et al.* 1988), seasonal partitioning of copulation-seeking and parental behaviours might not be in the males' best interest, and males with the attributes of T-males might be favoured.

The point we stress is that treatment with testosterone,

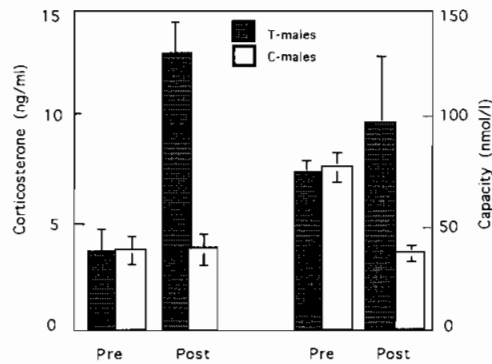


Figure 10. Corticosterone (ng/ml) and the capacity of the plasma to bind corticosterone (nmoles/l, a measure of corticosterone binding protein [CBG]), in intact, captive male Dark-eyed Juncos housed singly. Measurements were made before (pre-) and after (post-) subjects were treated with either an empty implant (C-males) or an implant packed with testosterone (T-males), and individuals were measured either for corticosterone or CBG but not both. Christensen *et al.* (unpubl.).

by affecting vocal behaviour, parental behaviour, use of space and sexual attractiveness, gives rise to interdependent consequences (Fig. 9). If we are to determine the net effect of these changes on fitness, we need to know the relative success of both T- and C-males in siring the young of both their social mates and the social mates of their neighbours, as well as their ability to protect their nests from predators and to contribute adequate nourishment to their young.

Effect of treatment with testosterone on physiology

Testosterone and lifespan

Several workers have anticipated how treatment with testosterone might affect male survival (Ligon *et al.* 1990, Zuk 1990, 1994, Zuk *et al.* 1990, Folstad & Karter 1992), and this raises the question of testosterone's effect on physiology.

We have earlier reported several physiological alterations that accompany treatment with testosterone and could influence male survival (summarized in Ketterson & Nolan 1992). First, males implanted with testosterone prior to the start of the breeding season quickly reduce their winter fat stores below those of controls (Ketterson *et al.* 1991b), a response that could be costly in the event that late spring snowstorms cover the food supply, as happens at their high breeding elevations. Second, corticosterone, the adrenal steroid, is found in higher concentrations in T-males than in controls, which could indicate T-males are under stress and/or are more subject to disease (Ketterson *et al.* 1991b). Third, depending on how long the T-implants remain in place, they either delay or suppress the pre-basic moult (Nolan *et al.* 1992), compromising overwinter survival (see below) and leaving the surviving birds whose moult was suppressed with

very worn plumage in the following year. Our recent efforts to relate testosterone and physiology have focused on corticosterone and the immune response.

Corticosterone

One possible explanation for elevated corticosterone in free-living T-males might be stress resulting from increased frequency of agonistic social encounters. However, this is speculation, because we have no field data on the frequency of encounters. To address this possibility indirectly, we administered testosterone to captives separated individually from one another in cages and thus unable to engage in normal agonistic encounters such as fighting. We found elevated corticosterone in these captive T-males, suggesting that the effect of testosterone on corticosterone in free-living T-males is not the result of aggressive interactions (L. Christensen *et al.*, unpubl.; Fig. 10). L. Christensen *et al.* (unpubl.) also found an increase in the capacity of the plasma of captive T-males to bind corticosterone, implying an increase in the concentration of corticosteroid binding globulin (CBG) (Fig. 10). This could mean that the observed testosterone-related elevation in circulating corticosterone does not represent an increase in *biologically active* corticosterone, but further study of this point is required (L. Christensen *et al.*, unpubl.). We are currently comparing the 'stress response' of T- and C-males (increase in corticosterone during handling), as described by Wingfield *et al.* (1992).

Fertility

The discovery of frequent extra-pair paternity in monogamous birds has led to an explosion of interest in sperm and sperm competition (e.g. Birkhead & Møller 1992, Gomendio & Roldan 1993, Baker & Bellis 1995), and there are many reasons to expect a relationship between testosterone and male fertility. From comparative studies we know that the testes of polygynous male birds are larger than those of monogamous species, and there is also an inverse relationship between testis size and male parental care (Møller 1988, 1989, 1991). Because the cost to females of incubating unfertilized eggs would be enormous, we would expect females to be responsive to any external indicators of relative fertility in males. Nevertheless, despite the obvious importance of sperm, relatively few studies have addressed individual variation in sperm density (Quay 1986, 1987, Birkhead & Møller 1992, Gomendio & Roldan 1993, E. Tuttle *et al.*, unpubl.), and none to our knowledge has investigated a relationship between testosterone and sperm reserves.

Testosterone is necessary to spermatogenesis (Epple & Stetson 1980, Ottinger 1983), so one reasonable suggestion is that elevated plasma testosterone might increase sperm production. However, the opposite prediction—that experimentally elevated testosterone could suppress spermatogenesis—is also reasonable. This is because, in addition to testosterone, spermatogenesis requires the gonadotropin,

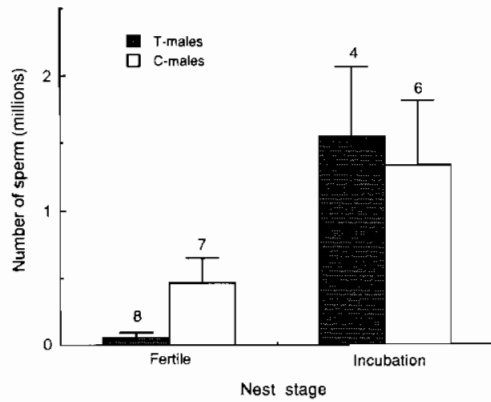


Figure 11. Testosterone and sperm/ejaculate in free-living Dark-eyed Juncos, according to treatment (T-males and C-males) and stage of reproduction. The X-axis indicates status of male's social mate: presumed fertile while building a nest or laying eggs, presumed infertile while incubating. Numbers of sperm in scientific notation, e.g. 2.00e+6 signifies 2 million. Data from T.L. Kast *et al.* (unpubl.).

follicle stimulating hormone (FSH), and the balance between testosterone and FSH is regulated by negative feedback (Johnson 1986). Hence, exogenous testosterone could interfere with spermatogenesis by suppressing FSH. (This background is necessarily simplistic because of its brevity and excludes important considerations, such as the ability of the testis to sequester testosterone and the role of inhibin; readers are referred to Bardin *et al.* [1988] and Jones & Lin [1993].) Finally, testosterone might also affect the quantity of sperm stored at any one time (sperm reserves) if it were to affect the rate of sperm utilization (copulation, Birkhead & Fletcher 1995). Copulatory behaviour is known to be dependent on testosterone's metabolites (Deviche & Schumacher 1982, Balthazart & Ball 1993), although its frequency need not be dose dependent.

To date, administration of exogenous testosterone has been variously reported to suppress, maintain, promote or have no effect on avian spermatogenesis (summarized in Lofts & Murton 1973, see also Turek *et al.* 1976, Desjardins & Turek 1977, Hagen & Dziuk 1985). Our implants have the effect of prolonging junco testosterone levels at their natural maximum rather than inducing levels higher than those found in nature (Ketterson & Nolan 1992), so it is not clear whether we should expect any effect on sperm production. Consequently, we are conducting a study of sperm reserves in free-living T- and C-males (T.L. Kast *et al.*, unpubl.). Kast manipulates the cloacal protuberance of male juncos and uses a microcapillary pipette to collect the ejaculate (Gee & Temple 1978, E. Tuttle, pers. comm.). Results to date suggest that sperm reserves may be somewhat depressed in T-males (Fig. 11). Whether this is attributable to diminished production of sperm or greater depletion of the reserves by higher rates of copulation is presently under study, but data to date are consistent with the likelihood of a testosterone-

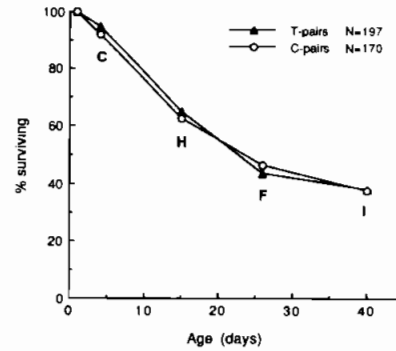


Figure 12. Testosterone and survival of eggs and young in Dark-eyed Juncos, 1989–1993. The figure presents nesting success according to male treatment, i.e. the proportion of nests in which at least one egg was laid that were still active at various stages of reproduction. C, clutch completion; H, hatching; F, fledging or nest-leaving; I, independence (respective sample sizes at start of laying indicated in legend).

induced enhancement of rate of copulation (compare Birkhead & Fletcher 1995).

Effect of treatment with testosterone on relative fitness

We present four sets of comparisons of fitness of C- and T-males. The first is apparent reproductive success, calculated on a brood-by-brood basis. For each nesting attempt, we tallied the number of young that hatched and survived to various ages, as well as the number of nesting attempts that ended in failure before fledging, usually because of predation. In the second, and with less complete information, we considered what we call realized reproductive success, which corrects apparent success for extra-pair fertilizations. A male may lose fitness because his social mate is fertilized by another male, and he may gain by fertilizing eggs laid by

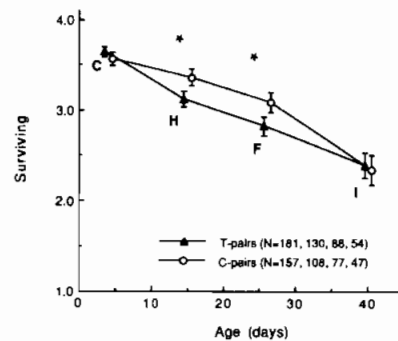


Figure 13. Testosterone and offspring production in Dark-eyed Juncos, 1989–1993. The figure presents mean numbers of eggs or young for broods surviving to various ages, according to male treatment and stage of reproduction. C, clutch completion; H, hatching; F, fledging or nest-leaving; I, independence. Sample sizes at these stages indicated in legend; asterisk indicates $P < 0.05$.

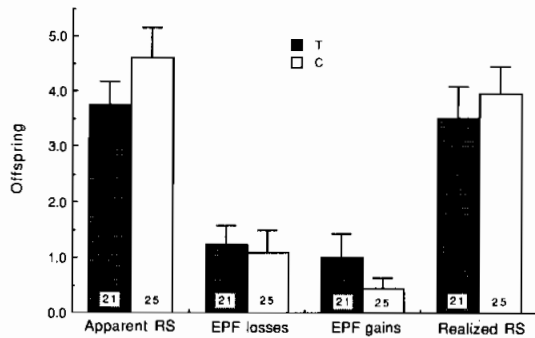


Figure 14. Testosterone and realized genetic reproductive success in Dark-eyed Juncos, 1990–1991. Results obtained using multi-locus DNA fingerprinting (by P.G. Parker). Apparent reproductive success indicates mean numbers of fledglings produced by social mates of T- and C-males during full breeding season. Some males of each treatment group lost paternity because other males sired young by means of extra-pair fertilizations (EPF); some males gained paternity by extra-pair fertilizations. Realized reproductive success adjusts apparent success by subtracting a male's extra-pair fertilization losses and adding his gains to show genetic reproductive success.

females that are socially mated to other males. In the third, we considered the possibility that T- or C-treatment affects a male's success not simply in the nesting attempt following implantation but also in future nest attempts. Such delayed effects on fitness might be revealed by the relative ability of males of the two treatment groups to retain their social mates and, if the mate is retained, the outcome of subsequent reproductive attempts. In the fourth, we looked for effects of treatment on male survival.

Apparent reproductive success

We have found no difference between T- and C-males in the numbers of offspring surviving to independence at approximately 28 days after hatching (Figs 12 and 13), although we observed interesting differences in relative success at earlier stages in the life of the young. Briefly, of 197 T-broods and 170 C-broods (1989–1993) associated with 100 individual T-males and 93 individual C-males, respectively, mean clutch size was very similar: 3.65 v 3.57 eggs (n.s.; T-males first in this and all subsequent comparisons), and percentage of nests lost to predation between clutch completion and hatching was nearly identical (29.9% v 29.4%, n.s.). Mean number of eggs that hatched differed significantly (3.12 v 3.36 nestlings, $P < 0.05$). Percentage of nests lost to predators between hatching and nest-leaving was greater in T-pairs, although not significantly so (21.3% v 15.9%, n.s.), and mean number of fledglings produced was significantly lower (2.82 v 3.08, $P < 0.02$).

Most studies of avian reproductive success do not consider the potentially important interval between nest-leaving and independence, but on many juncos' territories it is possible to monitor survival of fledglings during the 2-week period

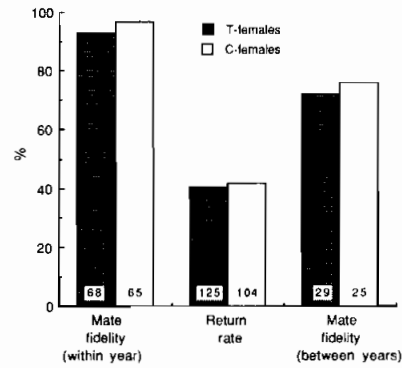


Figure 15. Testosterone treatment of males of Dark-eyed Juncos and its effect on indices of female fidelity within and between breeding seasons and survival (return from one breeding season to the next). Females were not treated with hormone and are classed according to the treatment of their mates.

in which they are still dependent on their parents. The percentages of entire broods that were lost during this period were similar (7.9% and 9.8%, n.s.), and the mean number of young reaching independence (2.39 v 2.34, n.s.) was virtually identical (compare Wolf *et al.* 1988). Because significantly more young left the nests of C-males, the observation that the number of young reaching independence is virtually identical indicates that the young of T-males were actually *more* likely to survive this last stage of parental care. Interestingly, our conclusion about the effect of treatment with testosterone on male reproductive success would have been different, and incorrect, if we had stopped monitoring success when the young left the nest. In sum, the net effect of phenotypic alterations induced by testosterone on apparent reproductive success appears to be nil when young are followed to independence.

Realized reproductive success

Obviously, realized or actual reproductive success is a more important measure of fitness than apparent reproductive success. To date, in collaboration with S.A. Raouf, we have extracted DNA from the blood of 46 males (21 T-males and 25 C-males), 43 females and 194 nestlings belonging to 69 broods. Samples were collected in 1990 and 1991. Using multi-locus DNA fingerprinting to determine genetic relatedness, we combined all broods produced by each male and his mate. Broken down by treatment, more broods of T-males than of C-males contained at least one young fathered by a neighbouring male (45% v 26%, n.s.). T-males were not the genetic sires of 33% of the young in the nests of their social mates, as compared with 23% loss of paternity for C-males (n.s.). Based on these same data (Fig. 14), the overall effect of testosterone on season-long realized reproductive success was as follows: Apparent mean reproductive success (numbers of nestlings/nest, regardless of relatedness) was lower in T- than in C-males (3.76 v 4.60 per male, n.s.), and the

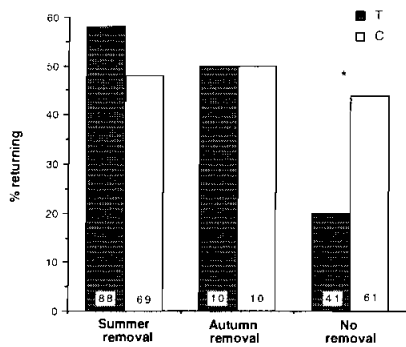


Figure 16. Testosterone and indices of survival in male Dark-eyed Juncos. Return rate from one breeding season to the next, according to time of year when testosterone implants were removed (if removed).

loss by T-males of young to extra-pair fertilizations was slightly greater (excluded nestlings in the male's own nest, 1.24 v 1.08). However, T-males sired slightly more young by extra-pair fertilization (gains of young in the nests of other males; 1.0 v 0.44, n.s.). The net effect of treatment on realized reproductive success (apparent success minus losses to extra-pair fertilizations plus gains by extra-pair fertilizations) was marginally lower success by T-males (3.52 v 3.96, n.s.). None of the differences reported in this paragraph was statistically significant, but the trends fit so closely with the observed behavioural differences of T- and C-males (less parental care, more time away from the nest, greater attractiveness to females) that we are in the process of analysing data from 1992–1994.

Delayed effects

We compared the masses at the time their young fledged of females mated to T- and C-males, but found no difference (mean of both treatments was 19.8 g, $P > 0.95$). Nor did we find a difference in the time elapsed between fledging of one brood and the laying of the first egg in the next nest (13.3 days v 12.9 days, $P > 0.84$) or in the size of the next clutch (3.49 eggs v 3.67 eggs, $P > 0.36$). We had predicted that females mated to T-males might be more likely to desert them between breeding attempts (because T-males had fed young less frequently), but this prediction was not borne out (Fig. 15; data updated from Ketterson & Nolan 1992). Annual return rates of females mated to T-males and C-males were quite similar (Fig. 15; data updated from Ketterson & Nolan 1992), and here too we had expected that mates of former T-males would be less likely to remate with them. Again, this prediction was not fulfilled (Fig. 15).

Male survival

Finally, we tracked the relative year-to-year return rates of males treated between 1987 and 1993 (C. Ziegenfus *et al.*, unpubl.; data updated from Nolan *et al.* 1992). T-males whose

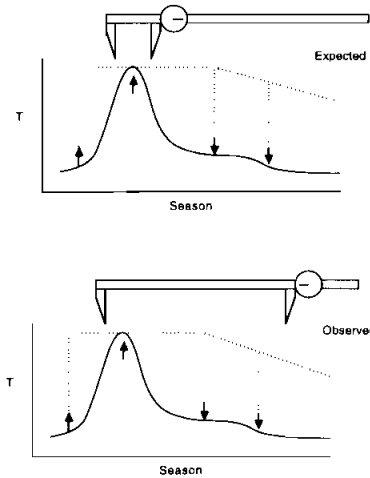


Figure 17. Consequences of deviations from normal seasonal pattern of testosterone secretion, stylized representation. Solid line presents plasma levels of testosterone in typical socially monogamous, temperate-zone passerines including juncos (Wingfield *et al.* 1987, Ketterson & Nolan 1992). Arrows represent times when testosterone implants were inserted (upward arrows) or removed (downward arrows) in the experiments reported herein. The effect of these manipulations was to extend the seasonal duration of elevated testosterone (effect of treatment represented by dotted line). Calipers appear in two settings. One (above) is closed tightly about the seasonal peak of testosterone and is designed to represent strong selection against phenotypes with deviant patterns. This is the *a priori* expected result. The second setting (below) is opened more widely to represent the relaxed selection on seasonal profiles that our studies have revealed thus far (the observed result).

implants were removed in late summer returned at a rate of 58% compared with 48% for C-males (n.s.; Fig. 16). Of those whose implants were removed in October, 50% returned, regardless of the original treatment (Fig. 16). Treatment affected rate of return from one breeding season to the next only when implants were left in place for the winter. In that case, testosterone significantly reduced return, which we interpret as survival (20% v 44%, $P < 0.05$; Fig. 16). We have speculated on the reasons for this difference (Nolan *et al.* 1992), which may include reduced insulation and possible loss of aerodynamic efficiency as the result of worn plumage. T-males may not fatten properly, or join flocks during the non-breeding season or move to lower ground in autumn. In any case, only when the time of exposure to testosterone was extended far beyond normal were we able to detect an effect of testosterone on our index of survival.

Summary of possible fitness consequences

Many of the phenotypic consequences of treatment with testosterone have potential consequences for reproductive fitness. Nevertheless, when we consider our data pooled over a 5-year period and confine our conclusions to differences that are statistically significant using conventional standards (type II error, $P < 0.05$, two-tailed), we find treatment with

testosterone depressed the number of offspring that hatched and fledged but not the number that reached independence, the latter being the more important index of success. We tentatively conclude that testosterone had no effect on apparent reproductive fitness, as determined on a brood-by-brood basis. We postpone any conclusion regarding realized reproductive success until we have analysed all our data.

Treatment with testosterone also depresses male return rate and thus probably male survival, but only if the implants remain so long that pre-basic moult is not simply delayed but is suppressed (Nolan *et al.* 1992). Figure 17 summarizes our interpretation of this result. The normal seasonal profile is of a typical socially monogamous passerine bird species and is depicted as a single early-season peak followed by a decline, although it is likely that males retain the capacity to elevate testosterone throughout the breeding season if conditions warrant it. The arrows in Figure 17 represent dates at which we manipulated testosterone, in one year to accelerate the peak (left-hand arrow) but usually to prolong its duration until implants were removed (right-hand arrows). The removal of implants in late summer (second arrow from right) returns the birds to the normal schedule. Once we delayed removing implants until autumn (arrow 4), and in some years we did not remove implants. Only in these last years was survival affected (recall Fig. 16).

A strict adaptationist would expect lower fitness among individuals whose testosterone profile deviates from the norm. Depending upon the intensity of selection (depicted with calipers in Fig. 17), we would have expected the costs to rise with the magnitude of the deviation. If the 'normal' profile is optimal and selection is intense, any deviation should be disadvantageous. We found that survival of T-males was not affected even when moult was delayed by some 4–6 weeks beyond its normal date (Fig. 17, arrow 4) and that it was significantly reduced only when we did not remove implants at all. We therefore suggest that the situation in the junco can be represented by the widely set calipers in the lower panel of Figure 17.

Another plausible view is that there are real differences in performance (fitness) between T- and C-males, other than the difference associated with suppressed moult, but our samples are too small and our measures too variable to reveal them. We have reported elsewhere (Ketterson & Nolan 1992, Ketterson *et al.* 1992) that the statistical power of many of our comparisons is low and we once again call attention to this limitation. It applies to most avian studies of natural selection in nature and leads some to suggest that tests of selection theory be reserved for organisms that can be studied in larger numbers, e.g. plants or invertebrate animals. If selection differentials between T- and C-males are on the order of 10%, we lack sufficient samples and power to detect them (Ketterson *et al.* 1992). Yet population genetic theory predicts that a variant that suffers sustained, or even occasional but consistent, differences in fitness of less than 1% would be eliminated (Wright 1968–1978).

Despite these caveats, we argue that avian studies of the kind reported here should continue. Even with their limi-

tations on numbers, the major advantages of field studies on birds are that so much of their behaviour is accessible and so many components of their fitness are quantifiable, from the laying of the eggs to the age of first reproduction. Thus it is possible to address some of the questions raised in the introduction to this paper, namely, the extent to which single traits are maintained at suboptimal levels because they are components of a trade-off, or the extent to which phenotypes are resilient enough to modify one trait to compensate for induced disadvantageous changes in another trait.

Finally, as we see it, it is important to continue to attempt to connect the study of mechanisms to the study of function. To understand the evolution of patterns of social organization, including mating systems and level of male parental effort, we must understand how animals assess their environments and mobilize appropriate behavioural and physiological responses. And if we are to understand what maintains existing distributions of phenotypes, we must know the consequences, in terms of fitness, for individuals that deviate from the norm. Hence we end this summary of our work to date as we have other summaries: this is a study in progress and we feel that firm conclusions will require more data.

FUTURE DIRECTIONS

We hope that this work will lead to a greater understanding of the mechanisms underlying mixed reproductive strategies (Trivers 1972). Now that ornithologists are aware of the common occurrence of extra-pair fertilizations in apparently monogamous birds (Gowaty & Karlin 1984, Westneat 1987, Westneat *et al.* 1990, Birkhead & Møller 1992, Møller & Birkhead 1993), we should expect males to have means of assessing the relative likelihood of reproductive success via mating effort as compared with parental effort, and we predict that their short-term (possibly day-to-day) hormonal and behavioural responses are likely to be plastic and adaptive. Thus, in circumstances in which mating effort is more likely to provide higher returns e.g. because nearby females are fertile, we might expect elevation of testosterone in males prepared to challenge for opportunities to inseminate them. In other circumstances e.g. when young have high reproductive value and/or are numerous, we might expect reduced secretion of testosterone and reduced mating effort, even though fertile females may be available nearby. Very little is known about mechanisms by which males might weigh stimuli associated with relative allocation of effort to mating and parental behaviours i.e. about the speed with which they can change allocation in response to changed circumstances. Thus a goal of our future research is to identify the physiological and behavioural responses of male Dark-eyed Juncos when changes in ephemeral circumstances shift the balance between the prospective benefits of mating and parental effort.

We especially thank our students and collaborators who graciously allowed us to refer to data as yet unpublished: L. Christensen, C. R. Chandler, D. Enstrom, A. Houtman, T. Kast, S. A. Raouf and J. Wolff. We are also grateful to our other collaborators, G. Ball, K.

Bruner, A. Dufty, S. Duncan, D. Sengelaub, W. Weathers and J. Wingfield, and to the post-doctoral associates, graduate students and research assistants who contributed so much to this project: L. Christensen, T. Johnson, T. Kast, S. A. Raouf, R. Titus, E. Tuttle, L. Wolf, P. Arguin, K. Bruner, L. Callahan, M. Chambers, D. Cristol, M. Crowe, D. Cullen, G. Gonzalez, J. Hill, J. Kidd, K. Kimber, J. C. Sanchez, D. Morris, S. Radjy, M. Ragland, A. Richards, S. Robbins, L. Rowe, E. Snajdr, M. Soenksen, J. Steele, M. Tavel and M. Watson. We thank E. Snajdr and K. Bruner for producing the figures. J. Wingfield, S. Pruett-Jones and L. Delph read the manuscript and made very helpful comments. Finally, thanks to our stimulating colleagues at Mountain Lake Biological Station and to those in our lab group, the Department of Biology and the Center for the Integrative Study of Animal Behavior at Indiana University.

REFERENCES

- Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. *Nature* 299: 818–820.
- Archawaranon, M. & Wiley, R.H. 1988. Control of aggression and dominance in White-throated Sparrows by testosterone and its metabolites. *Horm. Behav.* 22: 497–517.
- Arnold, A. 1982. Neural control of passerine song. In Kroodsma, D.E., Miller, D.H. & Ouellet, H. (eds) *Acoustic Communication in Birds*, Vol. 1: 75–94. New York: Academic Press.
- Baker, R.R. & Bellis, M.S. 1995. *Human Sperm Competition: Copulation, masturbation and infidelity*. London: Chapman & Hall.
- Ball, G.F. 1991. Endocrine mechanisms and the evolution of avian parental care. *Proc. Int. Orn. Congr.* XX: 984–991.
- Balthazart, J. 1983. Hormonal correlates of behavior. In Farner, D.S., King, J.R. & Parkes, K.C. (eds) *Avian Biology*, Vol. 7: 221–365. New York: Academic Press.
- Balthazart, J. & Ball, G.F. 1993. Neurochemical differences in two steroid-sensitive areas mediating reproductive behaviors. In Gilles, R. (ed.) *Advances in Comparative and Environmental Physiology*: 133–161. New York: Springer-Verlag.
- Bardin, C.W., Cheng, C. Y., Musto, N.A. & Gunsalus, G.L. 1988. The sertoli cell. In Knobil, E. & Neill, J.D. (eds) *The Physiology of Reproduction*, Vol. 1: 933–974. New York: Raven Press.
- Beletsky, L.D., Orians, G.H. & Wingfield, J.C. 1990. Effects of exogenous androgen and antiandrogen on territorial and nonterritorial Red-winged Blackbirds (Aves: Icterinae). *Ethology* 85: 58–72.
- Birkhead, T.R. & Fletcher, F. 1995. Depletion determines sperm numbers in male Zebra Finches. *Anim. Behav.* 49: 451–456.
- Birkhead, T.R. & Møller, A.P. 1992. *Sperm Competition in Birds: Evolutionary causes and consequences*. London: Academic Press.
- Boag, D.A. 1982. How dominance status of adult Japanese Quail influences the viability and dominance status of their offspring. *Can. J. Zool.* 60: 1885–1891.
- Burley, N.T. 1986. Sexual selection for aesthetic traits in species with biparental care. *Am. Nat.* 127: 415–445.
- Chandler, C.R., Ketterson, E.D. & Nolan, V., Jr. 1995. Spatial aspects of roost site selection in breeding male Dark-eyed Juncos. *Condor* 97: 279–282.
- Chandler, C.R., Ketterson, E.D., Nolan, V., Jr. & Ziegenfus, C. 1994. Effects of testosterone on spatial activity in free-ranging male Dark-eyed Juncos, *Junco hyemalis*. *Anim. Behav.* 47: 1445–1455.
- Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum Assoc.
- Clutton-Brock, T.H. 1991. *The Evolution of Parental Care*. Princeton, N. J.: Princeton University Press.
- Cunningham, D.L. & Siegel, P.B. 1978. Response to bidirectional and reverse selection for mating behavior in Japanese Quail *Coturnix japonica*. *Behav. Genet.* 8: 387–397.
- Desjardins, C. & Turek, F.W. 1977. Effects of testosterone on spermatogenesis and luteinizing hormone release in Japanese Quail. *Gen. Comp. Endocrinol.* 33: 293–303.
- Deviche, P. & Schumacher, M. 1982. Behavioural and morphological dose-response of testosterone and 5 alpha-dihydrotestosterone in the castrated male Japanese Quail. *Behav. Proc.* 7: 107–121.
- Dufty, A.M., Jr. 1989. Testosterone and survival: A cost of aggressiveness? *Horm. Behav.* 23: 185–193.
- Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* 197: 215–223.
- Endler, J.A. 1995. Multiple-trait co-evolution and environmental gradients in guppies. *Trends Ecol. Evol.* 10: 22–29.
- Epple, A. & Stetson, M.H. (eds). 1980. *Avian Endocrinology*. New York: Academic Press.
- Falconer, D.S. 1989. *Introduction to Quantitative Genetics*, 3rd ed. New York: Wiley.
- Feuerbacher, I. & Prinzing, R. 1981. The effects of the male sex-hormone testosterone on body temperature and energy metabolism in male Japanese Quail (*Coturnix japonica*). *Comp. Biochem. Physiol.* 70A: 247–250.
- Folstad, I. & Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139: 603–622.
- Fox, S.F. 1983. Fitness, home-range quality, and aggression in *Utastansburiana*. In Huey, R.B., Pianka, E.R. & Schoener, T.W. (eds) *Lizard Ecology: Studies of a model organism*: 149–168. Cambridge, Mass.: Harvard University Press.
- Freeberg, T.M., King, A.P. & West, M.J. In press. Social malleability in cowbirds (*Molothrus ater artemisiae*): Species and mate recognition in the first two years of life. *J. Comp. Psychol.*
- Gee, G.F. & Temple, S.A. 1978. Artificial insemination for breeding non-domestic birds. In McLaren, A. (ed.) *Symp. Zool. Soc. Lond.* 43: 51–72.
- Glickman, S.E., Frank, L.G., Holekamp, K.E., Smale, L. & Licht, P. 1993. Costs and benefits of 'androgenization' in the female Spotted Hyena: The natural selection of physiological mechanisms. *Perspect. Ethol.* 10: 87–117.
- Gomendio, M. & Roldan, E.R.S. 1993. Mechanisms of sperm competition: Linking physiology and behavioral ecology. *Trends Ecol. Evol.* 8: 95–100.
- Gowaty, P.A. & Karlin, A.A. 1984. Multiple maternity and paternity in single broods of apparently monogamous Eastern Bluebirds (*Sialia sialis*). *Behav. Ecol. Sociobiol.* 15: 91–95.
- Gyger, M., Karakashian, S.J., Dufty, A.M. & Marler, P. 1988. Alarm signals in birds: The role of testosterone. *Horm. Behav.* 22: 305–314.
- Hagen, D.R. & Dziuk, D.J. 1985. The effect of exogenous testosterone on homospermic and heterospermic fertility in the cock. *Biol. Reprod.* 32: 1080–1086.
- Hannslar, I. & Prinzing, R. 1979. The influence of the sex hormone testosterone on body temperature and metabolism of Japanese Quail. *Experientia* 35: 509–510.
- Harding, C.F., Walters, M.J., Collado, D. & Sheridan, K. 1988. Hormonal specificity and activation of social behavior in male Red-winged Blackbirds. *Horm. Behav.* 22: 402–418.
- Hegner, R.E. & Wingfield, J.C. 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male House Sparrows. *Auk* 104: 462–469.
- Johnson, A.L. 1986. Reproduction in the male. In Sturkie, P.D. (ed.) *Avian Physiology*: 432–451. New York: Springer-Verlag.

- Jones, R.C. & Lin, M. 1993. Spermatogenesis in birds. *In* Milligan, S.R. (ed.), *Oxford Reviews of Reproductive Biology* 15: 233–264. Oxford: Oxford University Press.
- Ketterson, E.D. & Nolan, V., Jr. 1992. Hormones and life histories: An integrative approach. *Am. Nat.* 140: S33–62.
- Ketterson, E.D. & Nolan, V., Jr. 1994. Male parental behavior in birds. *Ann. Rev. Ecol. Syst.* 25: 601–628.
- Ketterson, E.D., Nolan, V., Jr., Wolf, L. & Ziegenfus, C. 1992. Testosterone and avian life histories: Effects of experimentally elevated testosterone on behavior and correlates of fitness in the Dark-eyed Junco (*Junco hyemalis*). *Am. Nat.* 140: 980–999.
- Ketterson, E.D., Nolan, V., Jr., Ziegenfus, C., Cullen, D.P., Cawthorn, M. & Wolf, L. 1991a. Non-breeding season attributes of male Dark-eyed Juncos that acquired breeding territories in their first year. *Proc. Int. Orn. Congr.* XXI: 1229–1239.
- Ketterson, E.D., Nolan, V., Jr., Wolf, L., Ziegenfus, C., Dufty, A.M., Ball, G.F. & Johnsen, T.S. 1991b. Testosterone and avian life histories: The effect of experimentally elevated testosterone on corticosterone and body mass in male Dark-eyed Juncos (*Junco hyemalis*). *Horm. Behav.* 25: 489–503.
- Knobil, E. & Neill, J.D. 1988. *The Physiology of Reproduction*, Vols 1 and 2. New York: Raven Press.
- Kroodsma, D.E., Miller, D.H. & Ouellet, H. (eds). 1982. *Acoustic Communication in Birds*, Vols 1 and 2. New York: Academic Press.
- Lake, P.E. 1981. Male genital organs. *In* King, A.S. & McLelland, J. (eds) *Form and Function in Birds*, Vol. 2: 1–61. London: Academic Press.
- Ligon, J.D., Thornhill, R., Zuk, M. & Johnson, K. 1990. Male–male competition, ornamentation and the role of testosterone in sexual selection in Red Jungle Fowl. *Anim. Behav.* 40: 367–373.
- Lofts, B. & Murton, R.K. 1973. Reproduction in birds. *In* Farner, D.S. & King, J.R. (eds) *Avian Biology*, Vol. 3: 1–107. New York: Academic Press.
- Marler, C.A. & Moore, M.C. 1988a. Energetic costs of increased aggression in testosterone-implanted males. *Am. Zool.* 28: 186A.
- Marler, C.A. & Moore, M.C. 1988b. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behav. Ecol. Sociobiol.* 23: 21–26.
- Marler, C.A. & Moore, M.C. 1989. Time and energy costs of aggression in testosterone-implanted free-living male Mountain Spiny Lizards (*Sceloporus jarrovi*). *Physiol. Zool.* 62: 1334–1350.
- Marler, C.A. & Moore, M.C. 1991. Supplementary feeding compensates for testosterone-induced costs of aggression in male Mountain Spiny Lizards (*Sceloporus jarrovi*). *Anim. Behav.* 42: 209–219.
- Massa, R. & Bottoni, L. 1987. Effect of steroidal hormones on locomotor activity of the male Chaffinch (*Fringilla coelebs* L.). *Monit. Zool. Ital.* 21: 69–76.
- Maxson, S.C., Shrenker, P. & Vigue, L.C. 1983. Genetics, hormones, and aggression. *In* Svare, B.S. (ed.) *Hormones and Aggressive Behavior*: 179–196. New York: Plenum Press.
- Maynard Smith, J. 1977. Parental investment: A prospective analysis. *Anim. Behav.* 25: 1–9.
- McDonald, M.V. 1989. Function of song in Scott's Seaside Sparrow, *Ammodramus maritimus peninsulae*. *Anim. Behav.* 38: 468–485.
- McKittrick, M.C. 1992. Phylogenetic analysis of avian parental care. *Auk* 109: 828–846.
- Metz, K.J. & Weatherhead, P.J. 1991. Color bands function as secondary sexual traits in male Red-winged Blackbirds. *Behav. Ecol. Sociobiol.* 28: 23–27.
- Metz, K.J. & Weatherhead, P.J. 1992. Seeing red: Uncovered coverable badges in Red-winged Blackbirds. *Anim. Behav.* 43: 223–229.
- Møller A.P. 1988. Testes size, ejaculate quality and sperm competition in birds. *Biol. J. Linn. Soc.* 33: 273–283.
- Møller, A.P. 1989. Ejaculate quality, testes size and sperm production in mammals. *Funct. Ecol.* 3: 91–96.
- Møller, A.P. 1991. Sperm competition, sperm depletion, paternal care, and relative testis size in birds. *Am. Nat.* 137: 882–906.
- Møller A.P. & Birkhead, T.R. 1993. Certainty of paternity covaries with paternal care in birds. *Behav. Ecol. Sociobiol.* 33: 261–268.
- Moore, M.C. 1991. Application of organization-activation theory to alternative male reproductive strategies: A review. *Horm. Behav.* 25: 154–179.
- Moss, R., Watson, A., Rothery, P. & Glennie, W. 1982. Inheritance of dominance and aggressiveness in captive Red Grouse *Lagopus lagopus scoticus*. *Aggr. Behav.* 8: 1–18.
- Nolan, V., Jr. 1978. *The Ecology and Behavior of the Prairie Warbler, Dendroica discolor*. Ornithological Monographs No. 26. Washington, D.C.: American Ornithologists Union.
- Nolan, V., Jr., Ketterson, E.D., Ziegenfus, C., Cullen, D.P. & Chandler, C.R. 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.
- Nowicki, S. & Ball, G.F. 1989. Testosterone induction of song in photosensitive and photorefractory male sparrows. *Horm. Behav.* 23: 514–525.
- Oring, L.W. 1982. Avian mating systems. *In* Farner, D.S., King, J.R. & Parkes, K.C. (eds) *Avian Biology*, Vol. 6: 1–92. New York: Academic Press.
- Oring, L.W., Fivizzani, A.J. & El Halawani, M.E. 1989. Testosterone-induced inhibition of incubation in the Spotted Sandpiper (*Actitis mecularia* [sic]). *Horm. Behav.* 23: 412–423.
- Ottinger, M.A. 1983. Hormonal control of reproductive behavior in the avian male. *Poultry Sci.* 62: 1690–1699.
- Owens, I.P.F. & Short, R.V. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends Ecol. Evol.* 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*). *Anim. Behav.* 20: 112–118.
- Quay, W.B. 1986. The sperm balls of passerine birds: Structure, timing, fates and functions in free-living populations. *Biol. Reprod.* 34(Suppl.): 68.
- Quay, W.B. 1987. Spontaneous continuous release of spermatozoa and its predawn surge in male passerine birds. *Gamete Res.* 16: 83–92.
- Roff, D.A. 1992. *The Evolution of Life Histories: Theory and analysis*. New York: Chapman & Hall.
- Røskaft, E. & Rohwer, S. 1987. An experimental study of the function of red epaulettes and the black body colour of male Red-winged Blackbirds. *Anim. Behav.* 35: 1070–1077.
- Runfeldt, S. & Wingfield, J.C. 1985. Experimentally prolonged sexual activity in female sparrows delays termination of reproductive activity in their untreated mates. *Anim. Behav.* 33: 403–410.
- Saino, N. & Møller, A.P. 1995. Testosterone correlates of mate guarding, singing and aggressive behaviour in male Barn Swallows, *Hirundo rustica*. *Anim. Behav.* 49: 465–472.
- Scanes, C.G. 1986. Pituitary gland. *In* Sturkie, P.D. (ed.) *Avian Physiology*: 383–402. New York: Springer-Verlag.
- Schleussner, G., Dittami, J.P. & Gwinner, E. 1985. Testosterone implants affect molt in male European Starlings, *Sturnus vulgaris*. *Physiol. Zool.* 58: 597–604.
- Schumacher, M. 1990. Rapid membrane effects of steroid hormones: An emerging concept in neuroendocrinology. *Trends Neurosci.* 13: 359–362.

- Sefton, A.E. & Siegel, P.B. 1975. Selection for mating ability in Japanese Quail. *Poultry Sci.* 54: 788–794.
- Silver, R., Andrews, H. & Ball, G.F. 1985. Paternal care in an ecological perspective: A quantitative analysis of avian subfamilies. *Amer. Zool.* 25: 823–840.
- Silverin, B. 1980. Effects of long-acting testosterone treatment on free-living Pied Flycatchers, *Ficedula hypoleuca*, during the breeding period. *Anim. Behav.* 28: 906–912.
- Silverin, B. 1991. Behavioral, hormonal, and morphological responses of free-living male Pied Flycatchers to estradiol treatment of their mates. *Horm. Behav.* 25: 38–56.
- Sinervo, B. 1993. The effect of offspring size on physiology and life history: manipulation of size using allometric engineering. *BioScience* 43: 210–218.
- Sinervo, B. & Huey, R. 1990. Allometric engineering: An experimental test of the causes of interpopulational differences in performance. *Science* 248: 1106–1109.
- Sinervo, B. & Licht, P. 1991a. Hormonal and physiological control of clutch size, egg size, and egg shape in Side-blotched Lizards (*Uta stansburiana*): Constraints on the evolution of lizard life histories. *J. Exp. Zool.* 257: 252–264.
- Sinervo, B. & Licht, P. 1991b. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. *Science* 252: 1300–1302.
- Smith, D.G. 1972. The role of the epaulets in the Red-winged Blackbird (*Agelaius phoeniceus*) social system. *Behaviour* 41: 251–268.
- Smith, D.G. 1976. An experimental analysis of the function of Red-winged Blackbird song. *Behaviour* 56: 136–156.
- Stearns, S.C. 1989. Trade-offs in life-history evolution. *Funct. Ecol.* 3: 259–268.
- Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford: Oxford University Press.
- Travis, J. 1989. The role of optimizing selection in natural populations. *Ann. Rev. Ecol. Syst.* 20: 279–296.
- Trivers, R.L. 1972. Parental investment and sexual selection. In Campbell, B. (ed.) *Sexual Selection and the Descent of Man*: 136–179. Chicago, Ill.: Aldine Publishing Co.
- Turck, F.W., Desjardins, C. & Menaker, M. 1976. Antigonadal and progonadal effects of testosterone in male House Sparrows. *Gen. Comp. Endocrinol.* 28: 395–402.
- Vehrencamp, S.L. & Bradbury, J.W. 1984. Mating systems and ecology. In Krebs, J.R. & Davies, N.B. (eds) *Behavioural Ecology: An evolutionary approach*: 215–223. Sunderland, Mass.: Sinauer Associates.
- Wada, M. 1981. Effects of photostimulation, castration, and testosterone replacement on daily patterns of calling and locomotor activity in Japanese Quail. *Horm. Behav.* 15: 270–281.
- Wada, M. 1982. Effects of sex steroids on calling, locomotor activity, and sexual behavior in castrated male Japanese Quail. *Horm. Behav.* 16: 147–157.
- Wada, M. 1986. Circadian rhythms of testosterone-dependent behaviors, crowing and locomotor activity, in male Japanese Quail. *J. Comp. Physiol. A* 158: 17–25.
- Wada, M., Ishii, S. & Scanes, C.G. (eds). 1990. *Endocrinology of Birds: Molecular to behavioral*. Tokyo: Japan Societies Press.
- Watson, A. & Parr, R. 1981. Hormone implants affecting territory size and aggressive and sexual behaviour in Red Grouse. *Ornis Scand.* 12: 55–61.
- Weatherhead, P.J., Metz, K.J., Bennett, G.F. & Irwin, R.E. 1993. Parasite faunas, testosterone and secondary sexual traits in male Red-winged Blackbirds. *Behav. Ecol. Sociobiol.* 33: 13–23.
- West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.* 20: 249–278.
- Westneat, D.F. 1987. Extra-pair copulations in a predominantly monogamous bird: Observations of behaviour. *Anim. Behav.* 35: 865–876.
- Westneat, D.F., Sherman, P. & Morton, M.L. 1990. The ecology and evolution of extra-pair copulations in birds. *Current Ornith.* 7: 331–369.
- Williams, G.C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11: 398–411.
- Williams, H., Kilander, K. & Sotanski, M.L. 1993. Untutored song, reproductive success and song learning. *Anim. Behav.* 45: 695–705.
- Wingfield, J.C. 1984. Androgens and mating systems: Testosterone-induced polygyny in normally monogamous birds. *Auk* 101: 665–671.
- Wingfield, J.C. 1994. Modulation of the adrenocortical response to stress in birds. *Perspect. Comp. Endocrinol.* 520–528.
- Wingfield, J.C. & Farner, D.S. 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein-binding. *Steroids* 26: 311–327.
- Wingfield, J.C. & Moore, M.C. 1988. Hormonal, social, and environmental factors in the reproductive biology of free-living male birds. In Crews, D. (ed.) *Psychobiology of Reproductive Behavior*: 149–175. Englewood Cliffs, N.J.: Prentice Hall.
- Wingfield, J.C., Smith, J.P. & Farner, D.S. 1982a. Endocrine responses of White-crowned Sparrows to environmental stress. *Condor* 84: 399–409.
- Wingfield, J.C., Vleck, C.M. & Moore, M.C. 1992. Seasonal changes in the adrenocortical response to stress in birds of the Sonoran Desert. *J. Exp. Zool.* 264: 419–428.
- Wingfield, J.C., Hegner, R.E., Dufty, A.M., Jr. & Ball, G.F. 1990. The “challenge hypothesis”: Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* 136: 829–846.
- Wingfield, J.C., Newmann, A., Hunt, G.L. & Farner, D.S. 1982b. Endocrine aspects of female–female pairing in the Western Gull (*Larus occidentalis wymani*). *Anim. Behav.* 30: 9–22.
- Wingfield, J.C., Ball, G.F., Dufty, A.M., Hegner, R.E. & Ramenofsky, M. 1987. Testosterone and aggression in birds. *Am. Sci.* 75: 602–608.
- Wolf, L., Ketterson, E.D. & Nolan, V., Jr. 1988. Paternal influence on growth and survival of Dark-eyed Junco young: Do parental males benefit? *Anim. Behav.* 36: 1601–1618.
- Wolf, L., Ketterson, E.D. & Nolan, V., Jr. 1990. Behavioural response of female Dark-eyed Juncos to the experimental removal of their mates: Implications for the evolution of male parental care. *Anim. Behav.* 39: 125–134.
- Wolf, L., Ketterson, E.D. & Nolan, V., Jr. 1991. Female condition and delayed benefits to males that provide parental care: A male removal study. *Auk* 108: 371–380.
- Wright, S. 1968–1978. *Evolution and the Genetics of Populations*, Vols 1–4. Chicago, Ill.: University of Chicago Press.
- Zuk, M. 1990. Reproductive strategies and disease susceptibility: An evolutionary viewpoint. *Parasitol. Today* 6: 231–233.
- Zuk, M. 1994. Immunology and the evolution of behavior. In Real, L.A. (ed.) *Behavioral Mechanisms in Evolutionary Ecology*: 354–368. Chicago, Ill.: University of Chicago Press.
- Zuk, M., Johnson, K., Thornhill, R. & Ligon, J.D. 1990. Parasites and male ornaments in free-ranging and captive Red Jungle Fowl. *Behaviour* 114: 232–248.