

# Forum

## The beneficial sexually transmitted microbe hypothesis of avian copulation

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*Several hypotheses have been proposed to explain why female birds either copulate repeatedly with a single mate or copulate with multiple partners even though only a single copulation may be sufficient to fertilize an entire clutch. We hypothesize that females may directly benefit from high frequencies of copulation and multiple copulation partners if they receive a cloacal inoculation of beneficial sexually transmitted microbes (STMs) that can either protect them against future encounters with pathogens and/or serve as therapy against present infections. Experiments in domestic animal production, wildlife rehabilitation, and clinical medicine indicate that inoculations of beneficial microbes derived from the indigenous microflora of hosts can lead to nutritional benefits, resistance to colonization by pathogens, the elimination of infection, and improved immune system functioning in recipients. Our hypothesis predicts greater copulatory rates when the probability of the transmission of beneficial microbes exceeds that of pathogens and when the positive effects of beneficial microbes on host fitness exceed the negative effects of pathogens. Patterns of copulatory behavior in birds suggest the potential utility of our hypothesis. We discuss our hypothesis in the context of observed patterns of copulation in birds and propose some ways to directly test our hypothesis. Information on the probabilities of transmission during copulation of beneficial and pathogenic microbes and their relative potencies in birds are needed to directly test the predictions of our hypothesis.*

A plethora of hypotheses (e.g., Birkhead and Møller, 1992; Birkhead et al., 1987; Hunter et al., 1993; Keller and Reeve, 1995; Lumpkin, 1981; Petrie, 1992) have been proposed to explain the variations in copulatory behavior both among and within species of birds (Birkhead et al., 1987). Despite a great deal of theoretical musing over the potential costs and benefits of copulations to females, including extrapair copulations (EPCs), the realized costs and benefits are not very clear (e.g., Birkhead and Møller, 1992; Birkhead et al., 1990; Hamilton, 1990; Kempnaers et al., 1992; Lifjeld et al., 1993; Møller, 1994; Wagner, 1991, 1992; Westneat et al., 1990). Direct empirical evidence supporting or contradicting any hypothesized benefit to females is lacking for most species.

We propose that the cloacal inoculation of beneficial sexually transmitted microbes (STMs; viruses, bacteria, fungi) is a previously ignored direct benefit to females of participating in copulations. Our hypothesis explains why female birds might pursue multiple copulations with their mates or EPCs with males of higher quality than their social mates, and is at least as parsimonious as theories that predict only indirect female benefits (e.g., “good genes”; Hamilton and Zuk, 1982, see below). Although our hypothesis is directed at birds, it may also apply to other animals.

The avian cloaca serves the dual functions of excretion and gamete transfer. Microbes may be readily transmitted from

males to females during copulation because intestinal microbes could become incorporated into an ejaculate (Sheldon, 1993). Thus, sexually transmitted diseases (STDs) may be important selective forces in the evolution of avian mate choice (Hamilton, 1990) and mating systems (Lombardo, 1998; Sheldon, 1993). But microbes can also have beneficial effects on host health (Herceg and Peterson, 1997; Hutchenson et al., 1991; Prescott et al., 1996; Savage, 1977; van der Waaij, 1989) and therefore on reproductive success. The benefits associated with the horizontal transmission of beneficial microbes may be a selective force helping to mold the evolution of mate choice and copulation behavior in birds.

A theory that posits that female birds seek multiple copulations with one or more partners in order to be inoculated with beneficial microbes must meet these requirements: (1) microbes can have beneficial effects, (2) birds transmit beneficial microbes during copulation, (3) the transmitted microbes produce beneficial effects in their recipients, and (4) the probability of transmission and potency of effect of beneficial microbes relative to that of potential pathogens influences copulatory behavior.

### Requirement 1: the beneficial effects of microbes

Studies in commercial animal husbandry, wildlife rehabilitation, and clinical medicine demonstrate the beneficial effects of microbes on their hosts. The beneficial effects of gastrointestinal microbes are well documented in commercial animal husbandry (for reviews see Fuller, 1989; Hutchenson et al., 1991). Newborn and juvenile domestic animals inoculated with beneficial microbes are, on average, less likely to harbor potentially pathogenic species, grow more rapidly, and better resist challenges by pathogens than are uninoculated individuals (Hutchenson et al., 1991).

The beneficial effects of microbes in wild birds is suggested by the use of adult saliva during the rehabilitation of chimney swifts (*Chaetura pelagica*; Kyle and Kyle, 1993). The adult saliva contained a variety of microbes. The saliva used in rehabilitation could come from any healthy adult swift. Nearly 100% of nestling swifts less than 6 days old died if given food lacking the adult saliva supplement, whereas nearly 100% of those fed food inoculated with saliva were rehabilitated and released (Kyle and Kyle, 1993).

Beneficial microbes have also been used as therapy against infection (e.g., Bruce and Reid, 1988; Gorbach et al., 1987). In humans, crude fecal suspensions obtained from healthy individuals and administered as enemas to sick individuals have been used effectively to treat enterocolitis caused by *Clostridium difficile* (Bowden et al., 1981; Schwann et al., 1984). In rhesus monkeys (*Macaca maculata*), vaginal *Escherichia coli* infections have been cured by treatment with direct intravaginal application of vaginal microbes obtained from healthy monkeys (Herthelius et al., 1989).

Many studies have demonstrated that (1) “normal” indigenous microbes are important in providing resistance to intestinal pathogens and controlling the populations of opportunistic bacteria in the digestive and urogenital tracts in humans (e.g., Agnew and Hillier, 1995; Bruce and Reid, 1988; Hillier and Holmes, 1990; Lidbeck and Nord, 1993; Redondo-Lopez et al., 1990; Savage, 1977; Sobel, 1990; van der Waaij et al., 1971;), other mammals (Savage, 1969), and birds (Fuller, 1973, 1989; Hutchenson et al., 1991; Nurmi and Rantala,

1973; Snoeyenbos et al., 1983; Weinack et al., 1981, 1982), and (2) there is a host genetic component to the development of the indigenous microflora (Stern et al., 1990; van der Waaij, 1989; van der Waaij et al., 1971).

Mechanisms by which females may receive therapeutic inoculations include (1) bacteriophagic viruses (e.g., Levin and Bull, 1996; Smith and Huggins, 1983), (2) less virulent strains of pathogens, which, in becoming established, limit the colonization abilities of more virulent strains (Sprunt and Leidy, 1988), and (3) beneficial microbes that produce bacteriocins lethal to already resident pathogenic strains (Daw and Falkner, 1996; Jack et al., 1991; Waters and Crosa, 1991).

The microbial population of a healthy individual consists of a mixture of beneficial and potentially pathogenic microbes that can cause illness and death if the balance between them is disrupted (Fuller, 1989; Herceg and Peterson, 1997; Prescott et al., 1996; Savage, 1977; van der Waaij, 1989). Physical and psychological stress can disrupt the protective microflora (Tannock, 1983), leading to increases in pathogen populations and subsequent pathology (Fuller, 1989). One general effect of stress is for protective *Lactobacilli* spp. to decrease and pathogenic coliforms to increase (Fuller, 1989; Tannock, 1983). In addition, the hormonal state arising from changes in diet (Scrimshaw et al., 1968) and reproductive condition may affect the ability of the immune system to combat pathogens (Folstad and Karter, 1992). Thus, for many birds it is likely that the rigors of migration, territory establishment and defense, ecological and sexual competition, and reproduction itself influence the ecological balance of the microbes of the gastrointestinal and urogenital tracts, making them more susceptible to pathogens (Tannock, 1983).

Reproduction may be especially stressful. For example, infections of the blood parasite *Haemoproteus* spp. and heterophile:lymphocyte ratios which indicate stress in birds were positively correlated with reproductive effort in great tits (*Parus major*; Ots and Horak, 1996). The fact that the ecological balance between microbes in the gastrointestinal and urogenital tracts can be disrupted by situations that are commonly encountered by wild birds establishes the conditions necessary for our hypothesis.

For adult birds, potential pathways for the acquisition of beneficial microbes include copulation, mate feeding, coprophagy (cf. Troyer, 1982), and/or cloaca-pecking (cf. Davies, 1983). Coprophagy may be an inefficient way to obtain beneficial microbes that are obligate anaerobes (e.g., most *Lactobacilli* spp.) (Topley, 1983; Bokkenheuser, 1993), favoring the evolution of direct interindividual transmission by copulation or mouth-to-mouth transfer. The most direct pathway would be via copulation because it minimizes the exposure of gastrointestinal and urogenital microbes to hostile aerobic environments.

#### Requirements 2 and 3: the sexual transmission of microbes and their effects

The existence of avian STDs (Lockhart et al., 1996; Sheldon, 1993; Stipkovits et al., 1986) is direct evidence that birds inoculate each other with microbes during copulation. Moreover, Perek et al. (1969) showed by experiment that male domestic cockerels with semen contaminated with bacteria infected the females with which they copulated.

For females to benefit from receiving cloacal inoculations of beneficial microbes, those microbes must become established in the gastrointestinal and/or urogenital tract. Corrier et al. (1991) showed that cloacal inoculation of turkey poults with beneficial microbes reduced the load of gastrointestinal *Salmonella seftenberg* after an oral challenge at 3 days of age. This finding shows that microbes introduced into the cloaca

can become established in the gastrointestinal tract and may outcompete pathogens already resident in the host. In birds, beneficial STMs introduced into the cloaca have a direct route into the intestines and urogenital system.

#### Requirement 4: copulatory behavior in relation to the benefits and risks of sexual transmission of microbes

Explanations for the adaptive significance of variation in copulatory behavior among different species (Birkhead et al., 1987), populations (e.g., synchronous versus asynchronous breeders) (Stutchbury and Morton, 1995), ecological communities (e.g., temperate versus tropical zones) (Stutchbury and Morton, 1995), and degrees of sociality (Møller and Birkhead, 1993) in birds have been proposed. However, none has directly considered the influence that STMs might have on copulatory behavior.

Female attempts to receive beneficial microbes may help explain why some female birds copulate outside of their fertile periods (e.g., Fitch and Shugart, 1984; Flood, 1985; Lombardo, 1986; Power and Doner, 1980; Quay, 1985, 1989; Wagner, 1991; Wolf, 1975). Moreover, it has been difficult to understand why many female birds copulate repeatedly with the same male or with multiple males (Birkhead and Møller, 1992; Hunter et al., 1993; Petrie, 1992) when only one or few ejaculates may provide enough sperm to fertilize all of a female's eggs (Adkins-Regan, 1995; Birkhead, 1988). Repeated inoculations of beneficial STMs may be a direct benefit of multiple copulations and may be necessary for female birds to receive inoculations large enough to produce benefits. In clinical situations and during domestic animal production, repeated inoculations of antibiotics and/or beneficial microbes are used to produce the desired prophylactic, therapeutic, or nutritional effects (Fuller, 1989; Hutcheson et al., 1991; Savage, 1969).

#### The beneficial STM hypothesis of copulation in birds

Our hypothesis is based upon the following assumptions:

1. In birds, large numbers of copulations are *not* necessary to fertilize all eggs (Adkins-Regan, 1995; Birkhead, 1988). Copulations in excess of the minimum number required for fertilization require explanation. Part of that explanation is probably that females seek the benefits of sperm competition (Birkhead and Møller, 1992; Keller and Reeve, 1995), but we also assume that females can be favored for seeking *any* beneficial component of male ejaculate, not just highly competitive sperm (Eberhard and Cordero, 1995).
2. Some birds in the local population carry both beneficial and pathogenic STMs, while others carry only one or the other, or neither (e.g., Brittingham et al., 1988; Calnek et al., 1991; Cooper et al., 1980; Flammer and Drewes, 1988; Fritz et al., 1992; Lombardo et al., 1996; Petrak, 1982). For example, of 30 tree swallow (*Tachycineta bicolor*) semen samples screened for microbes in 1998, 11 (37%) were negative, 11 (37%) contained both beneficial (e.g., *Lactobacilli* spp.) and potentially pathogenic (e.g., *Salmonella* spp., *E. coli*) microbes, 1 (3%) contained only beneficial microbes, and 7 (23%) contained only potentially pathogenic microbes (Lombardo and Thorpe, unpublished data).
3. Beneficial STMs increase the health and vigor of their recipients, enhance host resistance to pathogenic STMs, and positively affect host reproductive success; pathogenic STMs have the opposite effects.

4. The probability that a female becomes colonized by STMs increases with the number of copulations she participates in (and/or partners she copulates with).
5. Birds will generally make risk-averting (*sensu* Kahneman et al., 1982) decisions (e.g., avoid partners infected with pathogenic STMs) while pursuing copulations because this will promote their survival and hence reproductive success. However, when their probability of survival has already been compromised by acquisition of pathogenic STMs, birds may be risk seeking (*sensu* Kahneman et al., 1982) to improve their odds of acquiring beneficial STMs as an antidote. Thus, ill females may increase their number of copulations/partner or number of partners even though this will inevitably also increase their chances of acquiring additional pathogenic STMs and thus further decrease their odds for survival and/or nesting success.

Given these assumptions, we hypothesize that females should pursue copulations to obtain STMs when these conditions obtain: (1) the probability of obtaining beneficial STMs exceeds that of obtaining pathogenic STMs, (2) the positive effects of beneficial STMs are greater than the negative effects of pathogenic STMs, and (3) the opportunity costs of obtaining copulations (i.e., the time, energy, and risk costs) are not too great for the female's budget. Additionally, if increased microbial diversity is beneficial, then mated females should pursue EPCs as well as copulations with their mates. However, the opportunity costs of EPCs will average higher because of mate guarding by the female's own mate, possible attack by the target male's female, and greater average distances between nonmated than mated individuals.

### The identification of potential partners

Positive correlations between the presence of beneficial STMs and host nutrition and resistance to pathogens (i.e., health) (Fuller, 1989; Herceg and Peterson, 1997; Hutcheson et al., 1991; Prescott et al., 1996; Savage, 1977; van der Waaij, 1989) strongly suggest a similar positive relationship between beneficial microbes and competitive ability. If carrying beneficial microbes is associated with good health (Fuller, 1989; Hutcheson et al., 1991; Savage, 1977; van der Waaij, 1989), then the ability of individuals to identify potential donors of beneficial STMs should be favored. Furthermore, if individuals that avoid copulating with carriers of pathogenic STMs have a selective advantage over those that do not, then the ability to detect infected partners by one sex would favor the ability to advertise freedom from disease by the other (Hamilton, 1990). Because there is a host genetic component to the establishment of symbiotic microbes (Stern et al., 1990; van der Waaij, 1989), carriers of strains of the most beneficial microbes will also simultaneously display their genetic quality.

An increasing body of empirical evidence suggests that well-developed secondary sexual characters may be reliable signals of health because they may be positively correlated with superior immunocompetence (*sensu* Folstad and Karter, 1992; Møller and Saino, 1994; Ros et al., 1997; Saino et al., 1995, 1997). Likewise, well-developed secondary sexual characters might also be reliable signals that an individual carries large numbers of and/or highly potent beneficial microbes. Therefore, female choice for showy males (Andersson, 1982; Møller, 1988; von Schantz et al., 1989) may have begun with females choosing the healthiest looking males as mates and EPC partners because they not only avoided STDs and other infectious diseases, but also received inoculations of superior beneficial STMs.

Our argument is parallel to the Hamilton and Zuk (1982)

“good genes” model of female choice, except that in our hypothesis females receive both direct and indirect benefits from choice. Our hypothesis and good genes models of choice are complementary, not mutually exclusive. The direct benefit of avoiding STDs and other infectious diseases by mating with showy males is implicit in Hamilton and Zuk (1982). Thus some current models of parasite-mediated sexual selection (Clayton, 1991; Hamilton, 1990; Hamilton and Zuk, 1982; Møller, 1994) may be useful in understanding the dynamics of choice for partners that are likely to transmit beneficial microbes. Good genes models (Hamilton and Zuk, 1982) may be applicable when females pursue copulations during their fertile periods, although health considerations should always be present. In contrast, if females pursue copulations outside of their fertile periods (e.g., during migration; cf. Quay, 1985) or when their indigenous microflora has been disturbed, then no assumptions about the genetic quality of potential partners is necessary (cf. Clayton, 1991), and our hypothesis is more applicable.

Both partners may be inoculated with STMs during copulation. However, because most birds lack an intromittant organ and ejaculates move from male to female, the transmission of microbes during copulation is likely to be asymmetrical with the probability of transmission from male to female being greater than that from female to male (Perek et al., 1969). Thus, while both sexes are favored for detection and signaling ability, because of the asymmetry of risk, females would be favored for greater detection ability and males for greater signaling ability. However, because males should avoid copulating with females infected with pathogenic STMs, females may be favored for signaling their freedom from infection (cf. Hamilton, 1990).

### Copulatory behavior in birds and tests of the hypothesis

Directly comparing the predictions of our hypothesis to data on avian copulatory behavior (see Birkhead et al., 1987; Birkhead and Møller, 1992, for reviews) is difficult because observations of copulation are biased in that they are most often of within-pair copulations during the breeding season. If females copulate to achieve fertilization, promote sperm competition, assess potential future partners, and acquire beneficial STMs, we have no a priori reason to predict patterns of copulatory behavior different from those already observed. However, our hypothesis also predicts that females pursue EPCs and copulations outside of their fertile periods to acquire beneficial STMs. Copulations performed solely to acquire beneficial STMs might be difficult to observe. Many more careful observations of EPCs and of birds outside of their breeding seasons are required before our hypothesis can be properly evaluated.

The observations that extrapair mating systems are more common among synchronously breeding songbirds than among asynchronously breeding songbirds and more common in the temperate zone than in the tropics (Stutchbury and Morton, 1995) are consistent with our hypothesis. Synchronous breeding and the short breeding seasons of the temperate zone limit the dispersal opportunities of microbes and thus favor the evolution of less virulent strains of pathogens (Ewald, 1994). We predict that copulations, including EPCs, will be more common when the probability of transmission of pathogenic microbes is low. In contrast, asynchronous breeding and the long breeding seasons of the tropics provide more dispersal opportunities for microbes and thus favor the evolution of more virulent strains of pathogens (Ewald, 1994). We predict that copulations, including EPCs, will be relatively uncommon when the probability of transmission of pathogenic microbes is high.

We also predict the evolution of female traits that facilitate colonization by beneficial microbes and impede colonization by pathogens obtained via copulation. First, females in a variety of bird species have the ability to selectively retain or expel ejaculates based on the identity of their copulatory partner (Adkins-Regan, 1995; Birkhead and Møller, 1992). Thus, we predict that females will be found to be able to retain or expel semen based on whether it contains beneficial or pathogenic microbes, respectively. Second, we predict the existence of mechanical and physiological impediments to colonization by pathogenic STMs in female reproductive tracts. For example, human females have a variety of defenses that help them avoid being colonized by pathogenic STMs (reviewed in Holmes et al., 1990; Profet, 1993). It is highly probable that females in all species with internal fertilization have evolved defense mechanisms to prevent colonization by pathogenic STMs.

Here we provide a short list of some ways to test our hypothesis:

1. Potential beneficial or pathogenic STMs can be identified by the association between their presence and/or abundance on host health, growth, social status, development of secondary sexual characters, and reproductive success.
2. The effects of experimental cloacal inoculation on individual health, growth, status, and expression of secondary sexual characters and on reproductive success could provide a direct way to identify both beneficial and pathogenic STMs.
3. Observations of the copulatory behavior of females of different ages could be used to determine whether younger females copulate more frequently with each partner and/or have more partners than older females on the grounds that younger females need to be "vaccinated" against future infections because of a lack of prior exposure to pathogens.
4. An experiment that induces copulations outside of female fertile periods by females that have been experimentally infected with pathogenic STMs could show that females pursue copulations as a way of acquiring beneficial STMs.

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## Energy budgets and risk-sensitive foraging in starlings

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*The effect of energy budget on risk-sensitive foraging was assessed in a laboratory experiment using starlings (Sturnus vulgaris). Subjects chose between two options offering the same mean amount of food per trial, but differing in variance: a "fixed" option gave 5 units food in every trial, and a "variable" option gave 2 or 11 units food with probabilities 2/3 and 1/3, respectively. We manipulated energy budgets by controlling the cumulative amount of food received by each bird at the end of a day. In one treatment (positive budget) individuals were allowed to eat at the level of their own ad-libitum daily consumption, while for the other (negative budget), food was rationed to provoke a steady drop in body weight during the experimental period. No subject was allowed to drop below 80% of its ad libitum body weight. Contrary to predictions from the "energy budget rule" and contrary to reported results of some other studies, starlings significantly preferred the "fixed" option irrespective of energy budget conditions. Our results support the view that persistent risk aversion for food amounts and risk proneness for food delays are the norm, and shifts in risk attitude according to energy budget are exceptions. Several algorithms, which may have evolved to maximize energetic pay off between variable food sources, can produce this trend as a side effect. We discuss two of these algorithms: (1) maximization of local (per trial) rate as opposed to global rate of gains, with longer handling time for larger rewards, and (2) choosing larger rewards and smaller delays subject to Weber's law in the memory for the parameters of each food supply.*

Virtually all actions result in outcomes with a degree of stochasticity. As a consequence, biological decision systems are likely to have evolved under the influence of outcome variance. This is particularly prevalent in the case of foraging behavior because food sources typically have different statistics (mean and variance in prey size, intercapture interval, rate of predator attacks, etc.), so that choices between food sources are actions with stochastic outcomes.

Living in a world offering statistically defined opportunities implies selection pressures on how to learn the statistics of the environment and how to use this knowledge in making choices. This last issue—namely, the choice between stochastic foraging sources differing in statistics which are known to the subject, is the target of risk sensitive foraging research and the topic of the experiment described here.

The most extensively developed theoretical framework in the functional analyses of risk is the collection of models jointly known as risk sensitive foraging theory (RSFT). The central tenet of RSFT is as follows: foraging success (rate of energy gain) results in fitness gains, but this relationship is unlikely to be linear. As a consequence, mean energetic gain from stochastic food sources is important, but not enough to rank their relative value; variances and skew also matter (Caraco and Chasin, 1984; McNamara and Houston, 1992). In a commonly considered scenario, a subject faces a single choice between two food sources that offer equal average gains but that differ in their variance. Fitness consequences are modeled as a step function resulting in death if the rate of energy gain resulting from the choice falls below a threshold and survival otherwise. The word "rate" here is used as the ratio of amount of energy gain divided by the time taken to gain it, so that prey sizes and temporal properties are both involved.

This simplified scenario is encapsulated in the so-called budget rule (Stephens, 1981). Under this rule, if the average payoff (common to both sources) is below the survival threshold, the least variable source will lead to lower fitness because it will have fewer chances of outcomes sufficiently above the mean to exceed the threshold and result in survival than the more variable alternative. The opposite will be true if the two sources have average payoffs above the survival threshold because the more variable source will have greater chances of yielding outcomes below the threshold. An animal is said to be in a positive budget if, on average, gains are above the survival threshold and on a negative budget if average gains are below that threshold. Hence, the prediction derived from the budget rule is that animals should be risk prone if they are on a negative budget and risk averse otherwise.

The budget rule poses both theoretical and experimental challenges because it is hard to determine when it should really apply. For instance, it is not obvious over which period the variance in outcome should be assessed. A simplified version of the theory refers to the outcome of a single choice, but single foraging choices rarely put animals below or above a threshold for survival. Indeed, it is virtually impossible to create this situation experimentally. Consequently, most experimental budget manipulations are based on a reasonable biological time period such as a 24-h cycle. If only one decision were to be considered, this ought to be a decision committing the animal for the full foraging day. This is, of course, hard to implement because with nonhuman subjects one cannot easily offer a choice committing the animal for such a long time and guarantee that the subject understands (is tuned to) the problem.

To overcome this difficulty, an additional, usually implicit, assumption is often made: if a subject's budget is manipulated over a 24-h period, its attitude toward risk will show in decision problems involving multiple, less consequential, choices. For instance, in the paradigmatic experimental tests of these ideas, Caraco and collaborators (Caraco, 1981; Caraco et al., 1980, 1990) modified the energy budget of small birds (dark-eyed and yellow-eyed juncos, *Junco* spp.) during experimental sessions lasting less than 4 h by appropriately setting the average size of rewards, the inter-reward interval, or the ambient temperature. This means that rate of gains during the session, if extrapolated to cover the whole day, would be below or above the average needs for survival. When the experimental sessions finished, the subjects were fed ad libitum. Birds in either a positive or a negative budget were then tested over multiple decisions within each session, by making them choose between food sources that differ in their variance (for instance, one source delivering always three seeds per choice and the other delivering one or five seeds per choice with equal probability). Because sessions involve forced trials to instruct the subjects on the statistical properties of the food sources, the overall variance in payoff among sessions is small, and it is impossible for the subject to attribute the variance in cumulative payoff over the whole session to the proportion of its choices. Predictions about risk attitude are based on assuming that the subjects extrapolate the variance in the consequences of their choices to the potential accumulated outcome over the 24-h period.

An additional problem is to guarantee that the subjects know the mean and variance of the stochastic alternative. The problem of assessing the parameters of a distribution using limited experience is a hard one in the best of cases, and much harder if the subjects do not know that they are dealing with two distributions of a given kind. Kacelnik and Bateson (1996) have shown that given the experienced sample sizes (the number of choices in a session and its consequences), good knowledge is unlikely in the case of the experimental

juncos; the confidence interval of the subjects' estimates must have been extremely large. Incomplete knowledge confounds the interpretation because under those conditions subjects are also expected to sample their alternatives so as to reduce uncertainty, whereas risk sensitivity predictions are developed under the assumption of full knowledge. Within the framework of these difficulties, it is, nonetheless, tantalizing that several studies have shown switches between risk aversion and risk seeking depending on energy budget (Caraco, 1981; Caraco et al., 1980, 1990). Should this result be confirmed, it may mean that animals modify risk attitude easily and generalize across time scales.

However, negative results are also often reported. In particular, risk attitude reversals have never been found in animals experiencing variance in the time or work components of foraging payoffs (see review in Kacelnik and Bateson, 1996). In a strong test of the theory, Ha et al. (1990) used gray jays (*Perisoreus canadensis*) that experienced variance in amount of work (and hence time) to obtain food rewards. The jays were subject to a budget that, in the negative treatment, led to their progressive weight loss over a number of days. In the positive budget condition they had enough food to hold their energetic reserves. The budget manipulation was thus stronger than in the junco tests, as the junco's budget was only controlled during the sessions, and they were allowed to hold their weights constant even in the negative budget treatments. In spite of this, the jays were consistently risk prone. A similar result is reported by Case et al. (1995) using water rewards.

Many differences between experimental conditions might account for inconsistent results. It may be that there is a fundamental difference between variance in amount and variance in time or effort. It may also be that the size of the subjects is crucial: perhaps smaller animals do switch in risk attitude, whereas larger species are persistently risk averse because of their greater ability to buffer short-term fluctuations in intake (students of human attitudes to risk usually refer to "risk aversion" rather than risk sensitivity, implying that aversion is the predominant attitude in their subjects). This account of results on the bases of the size effect sits uncomfortably with the observation that animals of different size show the same trends when comparing between amounts and delays, and even large species are systematically risk prone for delays.

It is also possible, however, that risk attitude reversals are much rarer and hard to replicate than previously thought (or even that success in previous studies was due to chance), a hypothesis that is hard to prove because it implies accepting a null hypothesis (lack of effect of budget manipulations). Indeed, it is likely that the set of reported failures to obtain a shift is a less complete representation of the number of attempts than is the case for positive results, which are easier to publish.

### Budget-insensitive interpretations

There are, of course, alternative budget-independent interpretations. Several models have been proposed that treat choices between variable sources of reward as cases of maximization of expected payoff in foraging, rather than reasoning about the putative nonlinearity of the gains-versus-fitness relation. All these models have in common the suggestion that something in the algorithm by which the subjects attempt to maximize feeding payoff leads to paradoxical choices (i.e., preference for submaximal average gain rate) in the presence of variance. Kacelnik and Bateson (1996, 1997) discuss these various models. These models per se do not predict any reversal in risk attitude as a function of the subject's state, but all the models could have features added to accommodate this

if evidence indicates that reversals are prevalent. These alternative models have been discussed elsewhere, so we restrict ourselves to their simple enumeration.

### Associative learning

Risk attitude could be a consequence of training. Because experimental animals are trained to choose among pecking keys, hopping perches, color lids, levers, etc., they must be exposed repeatedly to these manipulanda and their consequences to learn what they mean. Training efficiency is a function of both reward size and temporal contiguity between onset of the opportunity and outcome, and neither of these effects is linear. If the positive effect of reward size on the strength of the association between a manipulandum and food is concave, and the negative effect of delay is convex (as normally observed; see Tarpy, 1997) then one expects a lower subjective value for a manipulandum, leading to variable rather than to fixed outcomes when variance is in amount and the opposite when variance is in delay. Quantitative predictions based on this idea are hard to formulate because they depend on the precise form of the curves describing amount and delay dependency, including estimates of asymptotic associative value. This requires full parametric studies of the acquisition process under various sizes of reward or delays, and this information is not available within the foraging literature. Indeed, we do not have this information for the experiment reported here.

### Rate computations

If subjects base their choices on an algorithm that computes mean rate as the average of amounts and times taken per event, rather than over a continuous period (that is, they commit the fallacy of averages), then one should expect risk proneness for variance in delay and risk neutrality in variance for amount (Bateson and Kacelnik, 1996; Gilliam et al., 1982; Templeton and Lawlor, 1981). This risk neutrality turns into risk aversion if, while delays to food are constant, handling time is proportional to reward size. In this case, amount variance may produce risk sensitivity piggybacking on the temporal effects (Caraco et al., 1992).

### Weber's law

Risk attitude can also derive from the processing of information about amounts and times (Bateson and Kacelnik, 1995b; Kacelnik and Brito e Abreu, 1998; Reborada and Kacelnik, 1991). The idea here is that subjects choose the food source that they recall as yielding a bigger reward or a shorter delay, but stimuli (e.g., prey size or interper intervals) are remembered with confidence intervals proportional to their magnitude. This psychophysical effect (a form of Weber's law) causes subjects to remember the probability distribution of outcomes of a variable food source with greater positive skew than the objective (experienced) distribution. For instance, if two food sources yield normally distributed outcomes with equal mean but different variance, the subject will represent them internally as distributions with equal mean, but with a smaller median for the more variable one. Choice criteria that are sensitive to skew (e.g., if choices are based on the medians rather than on the means of internal representations) produce risk aversion for variability in amount (or any positive dimension of reward) and risk proneness for variability in time (or any aversive dimension of reward).

The goal of this paper is to reexamine the contrast between the reversal in risk attitude observed for amounts in juncos and the picture of weak risk aversion for amounts and strong risk proneness for delays emerging from other cases. We did not investigate delay variance here, as this is sufficiently well established, but we used a strong budget manipulation similar

to that used by Ha et al. (1990), exposing starlings to a choice between food sources that differ in variance in reward size as in the junco experiments. We also relate our results to budget-insensitive models.

## METHODS

### Subjects

The subjects were eight naive, wild-caught European starlings (*Sturnus vulgaris*). After capture the subjects were kept together for approximately 6 weeks in an outdoor aviary with free access to water and food, a mixture of turkey starter crumbs, Orlux pellets, and mealworms (*Tenebrio* sp.). One week before the beginning of the experiment, we moved the birds to an indoor laboratory and housed them in individual cages measuring 77 cm×50 cm×53 cm.

### Apparatus

The experiments were conducted in the birds' home cages. Each cage had a removable panel with a centrally mounted food hopper (4 cm×3.5 cm) and three response keys with 3 cm diameter (one at the center above the hopper, 23 cm from the floor, and the other two at 20 cm from the floor and 8 cm to the left and right of the hopper). The keys could be illuminated with either orange, green, or red lights. In front of the panel, at 20 cm from the floor, there was a perch from which the birds could access the hopper and the keys. This perch was mounted on a digital balance (Mettler 601), so that the bird's weight could be recorded without disturbance. An Acorn Risc PC 600 microcomputer programmed with Arachnid experimental control language (Paul Fray Ltd., Cambridge, UK) controlled the stimulus events and the response contingencies and recorded the data (including the readings from the balances). Birds' responses were reinforced with turkey starter crumbs delivered in the food hopper by pellet dispensers (Campden Instruments). Units of crumbs delivered from these dispensers had a mean±SD weight of 0.017±0.0048 g, and food rewards consisted of multiple units that were delivered at a rate of 1 unit/s.

Temperature in the laboratory ranged from 9°C to 15°C, and the lights were on between 0700 h and 1700 h. During the experiment the birds were visually but not acoustically isolated.

### Training

After 1 week of adaptation to the cages, the birds were induced to peck the response keys by a standard autoshaping procedure. Starlings initially experienced the delivery of standard rewards (5 units of food) preceded by 8 s of an orange light on the center key, with an intertrial interval (ITI) of 60 s. They were then gradually shifted to an operant schedule where rewards were delivered conditional on key pecking at the central key. During training subjects experienced 2 or 3 sessions per day of 100 trials each, and we provided ad libitum food after the last session.

### Energy budgets

#### *Ad libitum consumption*

We measured the daily ad libitum food consumption of each individual subject in our apparatus using a schedule in which birds had to peck once at an illuminated key to access food. A session of 450 trials began every day at 0700 h (lights on). In each trial an orange light illuminated the center key for up to 8 s, and pecking would cause the light to go off and the

**Table 1**

**Mean daily food requirement (grams of turkey crumbs) and free-feeding weight of the subjects**

Bird	Sex	Daily food intake (g) (mean ± SD)	Free-feeding weight (g) (mean ± SD)
0	Male	22.0 ± 0.96	73.5 ± 2.36
1	Female	18.5 ± 0.97	91.0 ± 4.69
2	Male	23.0 ± 2.15	79.5 ± 2.95
3	Female	18.5 ± 1.54	93.0 ± 1.86
4	Female	22.5 ± 1.18	75.5 ± 0.85
5	Female	21.0 ± 1.45	89.0 ± 3.19
6	Male	18.0 ± 0.87	81.5 ± 2.85
7	Male	23.5 ± 2.05	90.5 ± 2.09
Mean ± SE		20.88 ± 0.79	84.19 ± 2.69

delivery of a standard reward (5 units) followed by an ITI of 60 s. If no peck occurred in the 8-s interval, the light was turned off and the ITI started. Therefore, our subjects had a chance to feed every 68 s, during the first 8.5 h of the 10-h day. This schedule allowed the birds to obtain a theoretical maximum of 38 g of food per day (450 trials×5 units×0.017 g), considerably more than their normal consumption of about 20 g (see Table 1).

Birds were kept on this schedule for 9 days, and they stabilized their daily intake after 3 days (stability was judged by visual observation). We estimated each bird's daily consumption using the records from the last 6 days. Also, during this stage we measured the body weight of the subjects on every trial and used these readings to estimate their average free-feeding weights.

#### *Budget manipulations*

The total food received by the subjects during the choice experiment (explained in next section) was not enough to satisfy their daily needs, and supplementary food had to be delivered after the experimental sessions. We manipulated energy budgets by controlling the amount of supplementary food given to each subject. Birds on treatment N (negative budget) received an amount of supplementary food so that the total amount of food received by the end of the day (food delivered in the experimental sessions + supplementary food) would add up to half of their daily individual ad libitum intake, while birds on treatment P (positive budget) were given their full daily ad libitum consumption. The decision on halving their ad libitum intake was made after pilot experiments showed that less drastic reductions did not cause reliable weight loss. Starlings have the ability to change food utilization and reduce expenditure so as to compensate for imposed variations in intake (Bautista et al., 1998).

During the choice experiment we regularly monitored the subjects' body weight. This was particularly important in the negative budget treatment because it was crucial to observe a steady decrease in the bird's body weight while avoiding a weight loss beyond the boundaries of natural variation in the wild (no bird was allowed to fall below 80% of its free feeding body weight).

Supplementary food was delivered in two parts: one-third after the first session and two-thirds after the end of the second session. This ensured that the birds were not satiated when the second session began (1400 h) and that they had time to eat the supplementary food delivered after the second session, before the lights were turned off in the laboratory (1700 h).



### Choice experiment

In the main phase of the experiment, the birds faced a choice between two options: a fixed option which always delivered 5 units of food, and a variable option that offered either 2 units of food (with 0.66 probability) or 11 units (with 0.33 probability). Therefore, both options offered the same mean reward (5 units), but one had no variance and the other had a coefficient of variation between trials of 0.86. The fixed and variable options were signaled by different colors on the lateral keys (red and green, balanced between subjects).

We used a discrete trials procedure with a variable ITI generated by a truncated geometric distribution with a mean of 57.5 s (minimum = 45 s, maximum = 105 s). There were two types of trials: forced trials and choice trials. Forced trials started with the illumination of the center key with an orange light and, after the bird pecked, this light extinguished and one of the side keys (randomly chosen) was illuminated by either a red or a green light (signaling either the fixed or the variable option). When the bird first pecked the illuminated side key, a 5-s delay started, and the number of pecks during this interval was counted. The first peck after the 5-s delay extinguished the light and caused the delivery of the reward (corresponding to the presented option), which was followed by a new ITI. The purpose of the forced trials was to provide the subjects with information on the two options, forcing them to sample both options the same number of times before the choice trials. It also helped to prevent side biases because both options were presented the same number of times on each side. Some costs of this procedure are that it reduces the effect of the birds' preferences on its experienced long-term outcomes and that it reduces the variance in total payoff across sessions.

Choice trials also started with an orange light in the center key, but after the bird pecked at it, the two side keys were illuminated, one with a green light and the other with a red light (sides randomly chosen for each trial). When the subject pecked one of the side keys, the other light was turned off and a 5-s delay was timed. The first peck after the 5 s elapsed caused the delivery of a reward.

In addition to proportion of choices for each option in the choice trials, we recorded two other measures of motivation from the data collected on forced trials: the latency to accept the presented option and the number of pecks during the 5-s delay to reward. Relative motivation gives an indication of preference.

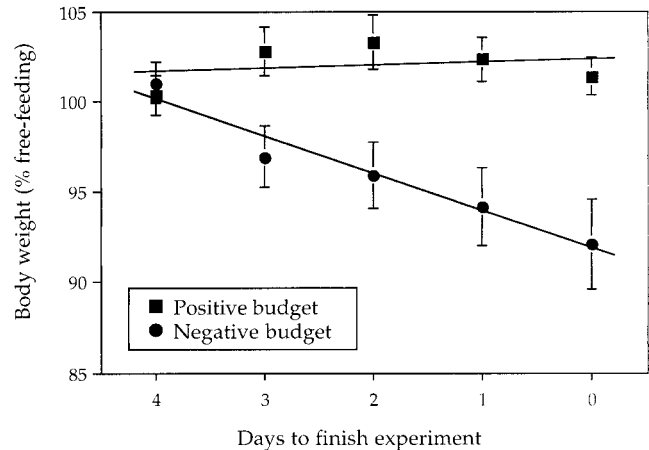
During the experiment there were two sessions per day, each consisting of 36 trials (the first session started at 0700 h and the second at 1400 h). Each session consisted of 3 consecutive blocks of 12 trials, in which the first 6 were forced trials and the last 6 were choice trials. Each option (fixed or variable) was always presented three times in the six forced trials of each block.

Subjects were divided in two groups of four, which were tested under both positive and negative energy budgets (treatments P and N). The experiment consisted of three stages, with one group following a N-P-N sequence of treatments and the other a P-N-P sequence. We interrupted each stage for all birds in both groups whenever the first of the birds on the negative budget treatment reached 80 % of its free-feeding weight (the first, second, and third stages lasted 6, 11, and 6 days, respectively). Between stages, birds were given 3 days of ad libitum food, during which no tests were run.

## RESULTS

### Energy budgets

Table 1 shows the estimated daily food intake and free-feeding weight of each subject. We calculated the ad libitum food in-



**Figure 1**

Mean  $\pm$  SE ( $n = 8$  subjects) body weight expressed as percentage of birds' free-feeding weights under the two treatments in the last 5 days of testing. Body weights decreased significantly on the negative budget treatment, but not on the positive budget treatment.

take (in grams of turkey crumbs) and the reference free-feeding weights by averaging the daily values recorded after day 3 of the energy budgets' assessment phase.

Figure 1 shows the effects of the two energy budget treatments on body weight in the last 5 days of each treatment. Birds in the negative budget treatment lost weight significantly (simple regression;  $R^2 = .24$ ,  $F_{1,38} = 12.2$ ,  $p = .001$ ,  $n = 8$  birds), and under the positive budget conditions they kept their body weights stable (simple regression;  $R^2 = .004$ ,  $F_{1,38} = 0.2$ ,  $p = .7$ ,  $n = 8$  birds).

### Risk preferences as expressed in choice trials

Figure 2 shows the proportion of choices for the variable option made by each subject under the two energy budget conditions. We calculated these values using all the choice trials of each individual, except those for the first 3 days of each stage. Data from the first 3 days were excluded to guarantee that enough time had elapsed for the energy budget treatment to have an effect.

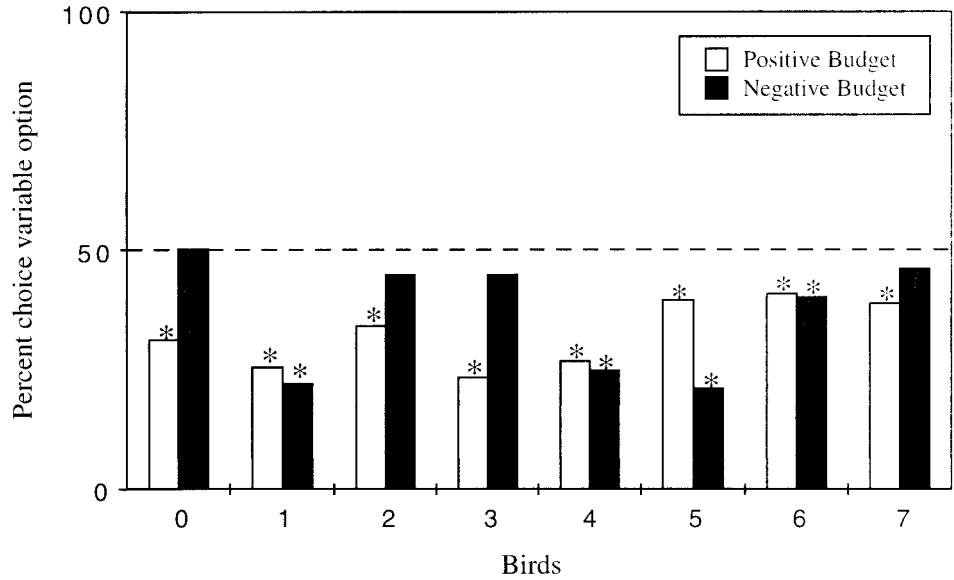
In general, birds were risk averse (Figure 2): four of them (birds 0, 2, 3, and 7) showed weaker risk aversion under the negative than under the positive energy budget; the opposite was observed in the remaining four. In treatment P all eight birds were significantly risk averse (binomial tests,  $p < .01$ ), whereas in treatment N risk aversion was significant only in four of the birds (binomial tests,  $p < .01$ ).

### Fit to theoretical models

To simplify the comparisons with predictions from various theories, we now examine population averages. We consider the predictive performance of four models, none of which, as we will show, resulted in a perfect fit. The comparison between average data and the predictions of the models is shown in Figure 3.

#### The budget rule

As a group, the subjects were risk averse (one-group  $t$  tests, two-tailed,  $t > 3.1$ ,  $p < .02$ ,  $n = 8$  birds) under both energy budget conditions (Figure 3). Our manipulation of energy budget did not affect starlings' preferences reliably because the difference between average percent of choice for the var-



**Figure 2**  
Percentage of choice for the variable option of each subject under the two budget conditions. Asterisks mark significant risk aversion (binomial tests,  $p < .01$ ).

variable option between treatments was not significant (paired  $t$  test, two-tailed,  $t = 0.9$ ,  $p > .4$ ,  $n = 8$  birds).

*Budget-insensitive models*

*Weber's law.* In Figure 3, the shaded area represents the predictions of the model of risky choice based on Weber's law with single sampling of the memory distributions (see Introduction and, for a detailed description, Kacelnik and Brito e Abreu, 1998). This model has one parameter that is assumed to be idiosyncratic among individuals, representing the coefficient of variation of the memory for each fixed percept. We calculated the predictions of this model using coefficients of variation ranging from 0.35 to 0.65, because these values cover the individual variation observed in starlings' coefficients of variation when assessing amounts of food in a similar set up (Bateson and Kacelnik, 1995a). The model predicts a percent

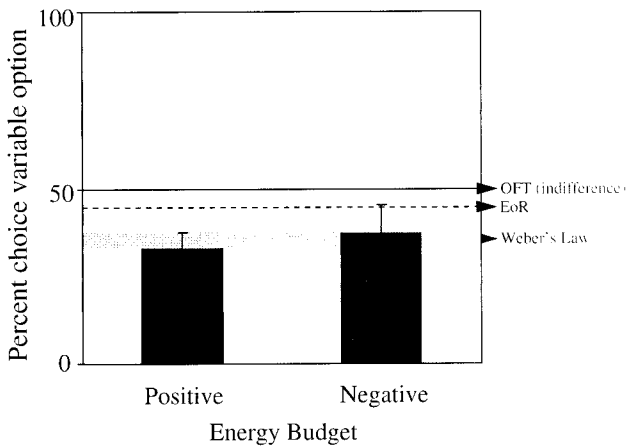
of choices for the variable option between 34.5% and 38.9%, a range that is inside the 95% confidence interval of our results (Figure 3). In spite of this remarkable fit, this must be seen with caution, as Figure 3 shows averaged data. Individual results showed considerable variation around the group's mean, as can be seen in Figure 2.

*Expectation of the ratios.* The dotted line marked with "EoR" in Figure 3 is the prediction of a variation of the local rate maximization model (Mazur, 1984; see further explanations in Bateson and Kacelnik, 1996). This model is based on assuming that subjects choose alternatives by computing rate of energy gain and choosing the highest score but that they compute rates with an algorithm that yields rate averages per trial rather than per session.

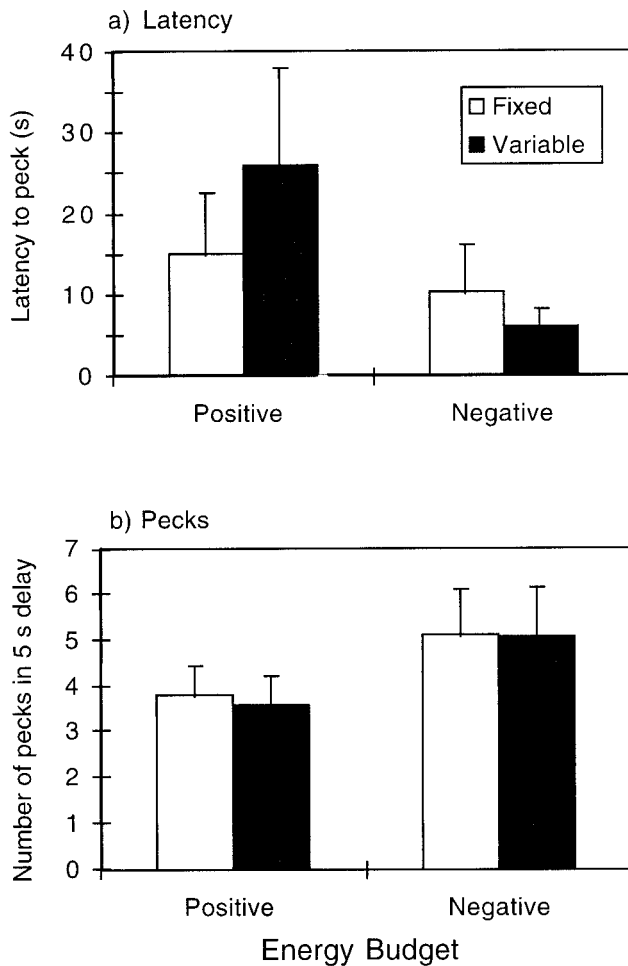
We implemented this model's predictions assuming that birds attribute an expected rate of gain to each option using only the time elapsed between their choice (pecking at the colored light) and the outcome. The two alternatives are valued according to the following equations:

$$V_f = \frac{F}{D + T_f} \quad V_v = \frac{\left( \frac{2S}{D + T_s} + \frac{L}{D + T_L} \right)}{3}$$

where  $V_x$  is the value attributed to the feeding option  $x$  (fixed or variable),  $F$  is the amount delivered in the fixed option (5 units),  $S$  and  $L$  are, respectively, the small and large amounts of the variable option (2 and 11 units),  $D$  is the delay between choice and reward (5 s), and  $T_x$  is the time required to deliver  $x$  units of food (1 s per unit). This computation excludes the times between each stimulus onset and choice. We analyze these latencies in forced trials in the next section, but we exclude them for the analysis of preference in choice trials because of the confounding factor that the latencies are chosen by the birds themselves. The local rate values according to these equations are 0.5 units/s for the fixed option, and 0.42 units/s for the variable option, and therefore according to this model one should expect exclusive preference for the fixed option. However, if the probability of a bird choosing an option is proportional to its attributed value (i.e., if starlings follow something close to the so-called matching law; Davison and McCarthy, 1988; Herrnstein, 1970), this algorithm can be used to predict partial preferences. The matching version of this model still predicts risk aversion, but it



**Figure 3**  
Mean percentage of choice of the eight subjects under the two budget conditions (error bars are 95% CIs). The two values are not significantly different from each other, but both are significantly different from 50%. The shaded area marks the predictions of the model based on Weber's law (Kacelnik and Brito e Abreu, 1998), the EoR dotted line marks the prediction of the local rate maximization model assuming matching (Bateson and Kacelnik, 1996), and OFT marks the prediction of classical rate-maximizing models.



**Figure 4**  
Mean  $\pm$  SE ( $n = 8$  birds) latency to peck (a) and number of pecks during the 5-s delay before the delivery of food (b) in forced trials for both the fixed and variable options, under the two energy budget treatments.

expects birds to choose the variable option in 45.7% of the trials  $[0.42/(0.42+0.5)]$ . Our results were clearly more extreme than this value in treatment P (i.e., risk aversion was stronger than predicted), but in treatment N the model's prediction falls within the 95% confidence interval of the mean percentage of choices for each option (Figure 3). Inclusion of the latencies would shift the model's prediction closer to risk neutrality (away from the data). In matching literature nomenclature, the subjects were "overmatching".

*Associative learning.* We referred in the Introduction to the idea that risk preferences may be due to the strength of association between stimulus and reward during training. We do not have sufficient information about the precise pattern of acquisition and asymptotic associative strength to make quantitative predictions on this basis, but the idea certainly applies to our experiment and is consistent with our results.

#### Risk preferences using latencies and number of pecks in forced trials

Figure 4 shows the average latency to peck and number of pecks during the 5-s delay to reward in the forced trials. Using the Box and Cox (1964) procedure, we estimated the best power transformation to normality ( $\lambda = -0.5$  for latencies,  $\lambda =$

0.23 for number of pecks) and performed a two-way repeated-measures ANOVA (treatment and option) on the transformed data. There was no significant interaction between treatment and option in the number of pecks; in both treatments birds pecked more when the fixed option was presented relative to the variable option ( $F_{1,7} = 10.76$ ,  $p = .001$ ). However, there was a significant interaction between treatment and option in the latency to peck ( $F_{1,7} = 3.94$ ,  $p = .047$ ). In treatment N latency to peck was shorter for the variable option than for the fixed option, whereas the opposite was true in treatment P. This interaction is the only aspect of our data showing motivational shifts in agreement with the budget rule.

As expected, energy budget affected subjects' motivation; latencies to peck were shorter and subjects pecked more times in treatment N than in treatment P. The effect on pecking rate is statistically reliable ( $F_{1,7} = 78.16$ ,  $p < .001$ ), but the effect on latencies cannot be reported because of the significant interaction between treatment and option.

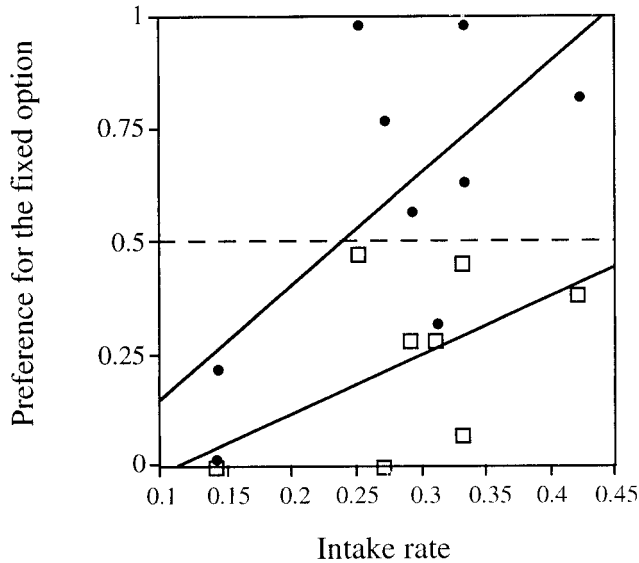
#### DISCUSSION

Our primary aim was to assess the effects of energy budget on starlings' preferences for variability in amount of food. We tested our subjects' preferences between a fixed feeding option (5 units of food) versus a variable option (2 or 11 units with probabilities 2/3 and 1/3, respectively), under both positive and negative energy budgets. The experiment was designed as a direct test of the budget rule (Stephens, 1981), which states that animals should be risk averse on positive energy budgets and risk prone on negative energy budgets. Contrary to these predictions, our subjects were significantly risk averse irrespective of energy budget conditions.

Starlings' risk-sensitive preferences have been tested before (Bateson and Kacelnik, 1995b; Bateson and Kacelnik, 1997; Reboreda and Kacelnik, 1991), and the usual result has been a strong tendency toward risk proneness when variability was caused by delay to food and weak risk aversion when variability was caused by amounts of food. A direct test of the energy budget rule, however, was unavailable.

In a related study, Koops and Giraldeau (1996) compared the use of two foraging tactics—"producer" (search for food) and "scrounger" (exploit food discovered by producers)—by starlings under conditions that differed in food availability (patch food density). These authors reported that, when food density was high, starlings increased the proportional use of scrounger, which is assumed to be a risk-averse tactic (because it decreases intake variance as well as intake rate), a result consistent with the general predictions of RSFT models. This study is hard to relate to ours because starlings may have responded to social rather than to energetic aspects of the situation. Social and energetic dimensions do interact in this species, to the extent that starlings do incur energetic losses in exchange for proximity to conspecifics (Vásquez, 1995).

One observation by Reboreda and Kacelnik (1991) suggested that there could be some effect of energy budget on starlings' preferences for risk. They tested starlings under two different treatments. In both treatments the birds faced a choice between a fixed food source and a food source with variable outcomes. They did not manipulate the energy budget directly, as their main goal was to compare risk preferences between amount and time variability. Their results confirmed the trend toward weak risk aversion for amount (which we now reaffirm) and strong risk proneness for delay. However, due to the way food was delivered, (reward amounts were controlled by time of access to a food hopper), some of the birds experienced higher rates of intake during the experiment than others because they were more efficient at scooping food. Using these interindividual differences, Reboreda



**Figure 5**

Data from Rebores and Kacelnik (1991) plotted and fitted with a regression line (circles, variability in amount; squares, variability in delay). There was a significant correlation between intake rate and risk aversion, as predicted by the energy budget rule (Stephens, 1981), both for variability in amount ( $r = .64$ ,  $p < .05$ ) and in delays ( $r = .61$ ,  $p < .05$ ).

and Kacelnik found a significant, positive correlation between rate of intake and risk aversion (Figure 5).

Although the budget effect was only a correlational observation, it suggested that energy budget could have an effect on starlings' risk preferences. However, under the more controlled conditions of the present test, the choice results failed to confirm this effect. We examined motivation toward fixed and variable rewards by looking at the delay in accepting rewards of either kind in forced trials. These latencies showed a significant interaction between variance and budget, in agreement with the budget rule.

In a recent study designed to separate the effects of variance from that of unpredictability in outcomes, Bateson and Kacelnik (1997) tested starlings' preferences for fixed versus variable delays to reward using a protocol that included a weak manipulation of energy budget (within-session budget was affected, but there was no loss of body weight during the experimental period). They found no effect of energy budget on preference, and, as in all the studies where risk is introduced by variability in delay, their subjects were strongly risk prone.

It is tempting to argue that failures of the budget rule may have been due to limitations of experimental design, such as insufficiently strong budget manipulations or insufficient difference in variance between alternatives. We do not think this line of thinking is promising, though. First, exactly the same caveats should apply to the experiments using juncos, where significant reversal of risk preferences were been obtained in spite of a positive overall budget. Second, in Bateson and Kacelnik's (1997) study the starlings were consistently risk prone, as it is always found with respect to delay, so that in any case risk bias was against the prediction of the budget rule for birds in a positive budget.

Because budget considerations fail to account for our results, we focus on how alternative, budget insensitive, interpretations relate to the data. Two aspects need to be examined. (1) Why is it that in spite of the strong logic of the energy budget rule, most animals seem not to have developed behavioral mechanisms that comply with it? (2) If RSFT were

to be abandoned, are there other theoretical accounts of how decision-making under uncertainty may have evolved?

The answer to the first question might be found in the analysis provided by McNamara (1996). He considered the relative performance of three strategies: optimal risk sensitive behavior (choose between fixed and variable options according to optimal state-dependency), rigid risk aversion (always prefer the fixed option), and rigid risk proneness (always prefer the more variable food supply). He performed his analysis only for variability in amount, but because this is the variable controlled in the present experiment, the results should apply. McNamara's fundamental finding is that although flexibility in risk attitude leads to higher fitness, under the majority of scenarios implemented in his simulations, rigid risk aversion led to a very small fitness loss, while rigid risk proneness would lead to large loss of fitness. This is mainly because the conditions favoring risk proneness are rare and normally extreme, and mortality in those cases would be high whatever the subjects choose to do. In addition, optimal implementation of flexible risk attitude assumes good knowledge of the statistical parameters of the food supply, but this assumption is rarely justified and perhaps almost unachievable in nature. With hindsight, it seems likely that the pressure to develop mechanisms for a change in risk bias according to budget may have been too weak to result in observable behavioral consequences, and what is puzzling is the fact that these reversals were often reported.

In an extensive review of studies on risk sensitivity, Kacelnik and Bateson (1996) point out that the experiments that reported a switch in preference as predicted by the energy budget rule were all on insects or relatively small fish, birds, and mammals (see also Hamm and Shettleworth, 1987). Studies on larger species such as rats, pigeons, starlings, and gray jays failed to find a shift in preference with the manipulation of energy budget. Kacelnik and Bateson suggest that body mass could be an important variable affecting risk sensitivity because smaller species with less reserves are more likely to have been subject to strong selection for short-fall minimization. Larger animals can keep a permanently higher relative level of body reserves, and therefore only rarely reach states in which a budget-dependent shift in risk sensitivity would affect their survival probability. This hypothesis can only be tested with a proper comparative study, which has not been done yet.

Second, if RSFT were to be left out, is there any other functional perspective that does a better predictive job? One possible answer is that choices between variable food sources may be better understood when looked from the perspective of maximization of rate of energetic payoff, rather than when examined from the point of view of strategic responses to variability per se. To accommodate the data, however, additional assumptions about the constraints faced by animals in computing average rates are necessary because typically they do not always choose so as to maximize long-term average rate of gain.

We compared our results against the predictions of two such models. In one of them, EoR ("expectation of the ratios", see Bateson and Kacelnik, 1996), subjects compute reward rate in both options and choose the better alternative, but their computation of rate is based on averaging the ratio of gain to time on a per-trial basis rather than on a per-time basis, and this leads to deviations from overall rate maximization. We took into account that larger rewards take longer to consume. This was certainly true in our set up because food units were delivered at 1-s intervals. This model admits various implementations, and although it correctly predicts risk aversion for our experiment, it requires ad-hoc modifications to deal with partial preferences.

The other model in this category (Weber's law and single

sampling) is based on considering the information-processing mechanisms involved in choice. The subjects remember the properties of each food supply with a degree of uncertainty and then make choices between samples taken from their memory for each source of reward, choosing always the sample that appears to lead to a better reward. The way uncertainty is included derives from what is known of perception of food amounts and time intervals. This model (Gibbon et al., 1988; Reboresda and Kacelnik, 1991) is described in full as applied to the present experiment and extended to more general cases by Kacelnik and Brito e Abreu (1998). It predicts risk aversion for desirable outcomes such as food amounts and risk proneness for aversive outcomes such as food delays. It also predicts partial preferences because in different trials sampling from memory yields different values for the two food supplies. We found a remarkable fit between the predictions of this model and the average results of our group of animals, but the fit is weaker when applied to each individual subject.

In conclusion, we found that starlings are persistently risk averse when amount variability is involved and that this is unaltered by energy-budget manipulations. These results, together with those of other studies showing that risk attitude for delays has the opposite sign and is equally resistant to budget manipulations, are consistent with the hypothesis that in natural environments maximization of mean rate of gain may be paramount, but that the mechanisms by which mean rates are computed may lead to paradoxical (not rate-maximizing) choices under experimental circumstances.

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## Stress, testosterone, and the immunoredistribution hypothesis

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Recent interest in parasites and sexual selection has focused attention on the paradox that the sexual displays which indicate parasite resistance in male vertebrates are triggered by testosterone, an apparently immunosuppressive hormone. We question the underlying assumption that testosterone is immunosuppressive and offer here the alternative of immunoredistribution to explain the changes in circulating leukocytes associated with male displays and elevated testosterone. First, we briefly examine three hypotheses that have attempted to resolve the testosterone immunosuppression paradox (Folstad and Karter, 1992; Hillgarth et al., 1997; Wedekind and Folstad, 1994). Although the immunoredistribution hypothesis under-

mines the premise of these hypotheses, there are other problems intrinsic to each one.

### The immunocompetence handicap hypothesis

Folstad and Karter (1992) proposed the immunocompetence handicap hypothesis as an extension of Zahavi's (1975) handicap hypothesis for the evolution of secondary sexual characteristics. While Folstad and Karter's hypothesis offered an explanation for higher parasite loads in males than in females, it has been used to explain both correlation and lack of correlation between testosterone and reduction in indices of immunity. Specifically, if males with high levels of testosterone have higher indices of immunity or lower parasite loads, the interpretation would be that those males have such high-quality immune systems that they can overcome the immunosuppression of testosterone (Zuk, 1996). On the other hand, if high-testosterone males have higher parasite loads, the interpretation would be that those males are of such high quality overall that they can display and attract females despite higher infection due to immunosuppression (Salvador et al., 1996; Weatherhead et al., 1993). And if no relationship is found between testosterone and parasite loads, the argument is that the high-quality males "are reliably signaling their resistance to parasites since they are still able to fend off parasites in the presence of high circulating levels of androgens" (Saino and Moller, 1994:1331). The invocation of the immunocompetence handicap to support such contradictory trends undermines the utility of the hypothesis.

### The resource allocation hypothesis

Wedekind and Folstad (1994) suggested that testosterone suppresses the immune system so that essential resources can be allocated instead to produce secondary sexual characteristics such as horns, songs, or stamina in repeatedly performing a display (Enstrom et al., 1997; Hunt et al., 1997). Although this would explain the adaptive trade-off underlying the immunocompetence handicap, it could also stand alone as an explanation for testosterone immunosuppression. However, Hillgarth and Wingfield (1997) pointed out that this explanation is unlikely to account for immunosuppression because the metabolic resources saved by suppressing immunity would be trivial compared to the associated risk of infection.

### The sperm protection hypothesis

Hillgarth et al. (1997) offered the alternative hypothesis that the immunosuppressive effect of testosterone protects haploid spermatozoa, which are antigenic because they are formed long after the development of the immune system. Despite the partial protection of spermatocytes by Sertoli cells and the blood-testis barrier, some lymphocytes can pass into the seminiferous tubule (Turek and Lipshultz, 1994). Hillgarth et al. (1997) suggest that testosterone suppresses antibody production in order to protect antigenic sperm. Thus, testosterone could pleiotropically reduce antibody production in general when it is in the systemic circulation to regulate other secondary sexual characteristics. Penn and Potts (1998) question the likelihood of this hypothesis because there would be strong selection against sensitivity of immune cells to testosterone outside the testes. Although the sperm protection hypothesis might account for some reduced antibody-mediated immunity in high-testosterone males (Folstad and Skarstein, 1997), it still does not explain the change in numbers of circulating leukocytes associated with elevated testosterone.

None of the current explanations for immunosuppression by testosterone is particularly satisfying because of the huge

selective cost of any significant impairment of immunity to pathogens. If immunity to parasites and disease is as important as Hamilton and Zuk's (1982) model suggests, suppression of immunity should be a rare and limited phenomenon. This has led us to question the underlying assumption that testosterone actually causes immunosuppression.

### The immunoredistribution alternative

New insights into the immune response to stress offer an alternative explanation for the correlation between high levels of testosterone and changes in the immune system. We propose that leukocytes are temporarily shunted to different compartments of the immune system in response to testosterone, as they are in response to other steroids. This process, called immunoredistribution, would be easy to confuse with immunosuppression if immunity is assessed by leukocyte counts or if the measure of immunity is sampled at only one time or in only one tissue.

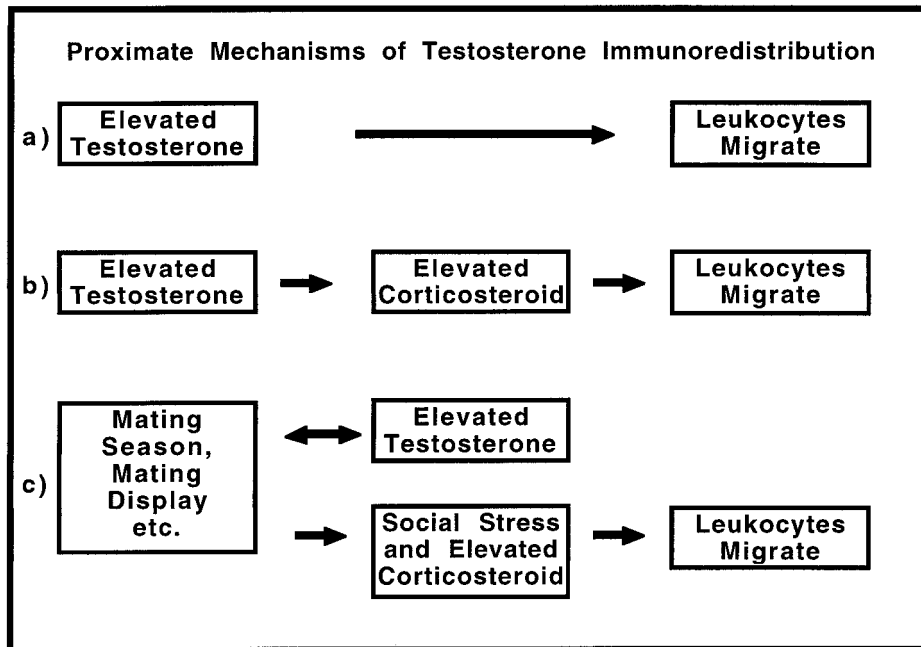
Unlike immunosuppression, redistribution is a temporary shifting of immune cells to compartments where they are likely to be more useful. This is far more than a semantic distinction. Immunosuppression implies that there is a single immune system which is inhibited from acting throughout the body and that lower cell counts result from elimination or reduced production of immune cells. Immunoredistribution is a quickly reversible relocation of immune cells to sites where they are most likely to be useful but perhaps less likely to be detected by researchers. Immunoredistribution is a well-documented response to stress and the associated elevation in circulating corticosteroids.

### Stress and immunosuppression

Although stress-induced immunosuppression is assumed to be a common phenomenon, there are a number of systematic problems with the evidence and interpretation of it. First, Keller et al. (1992) point out that few of the studies that describe inhibition of immune cell function controlled for changes in the proportions of different types of immune cells, and thus may not have been measuring inhibition at all. Equally important is the empirical evidence that the endocrine and immune responses to stress are complex and depend on a number of factors, including the sex ratio and social composition of the group under study (Sapolsky, 1986; Taylor et al., 1987). These factors are typically not controlled or taken into account. When they have been controlled, no suppression of immune cell activity was found (Klein et al., 1992). In addition, acute and chronic triggers are often lumped together. Despite these problems and the fact that responses may differ in different species, we do not question the evidence of suppression of immune cell activity under pharmacological doses of steroids. However, these examples only demonstrate that the activity of immune cells can be artificially suppressed, not that suppression is a normal physiological response to corticosteroids or testosterone.

### Stress and immunoredistribution

Immunoredistribution is an alternative explanation for the apparent immunosuppressive effect of stress. Environmental stress and social stress are both known to increase the level of circulating corticosteroids which in turn affect the immune system (Harbuz and Lightman, 1992; Morrow-Tesch et al., 1994; Taylor et al., 1987). This common mechanism is due to activation of the hypothalamus-hypophysis-adrenal axis. In response to the elevated corticosteroid levels, leukocytes exit peripheral blood circulation and enter lymph nodes, skin, and



**Figure 1**  
In addition to the two mechanisms by which testosterone is likely to trigger immunoredistribution (a and b), it is also possible that increases in testosterone correlate with redistribution but are not a direct cause of the phenomenon (c).

other tissues where they are well positioned to combat challenges from new trauma (Dhabhar, 1998; Dhabhar and McEwen, 1996). Circulating leukocytes then return to normal levels within a few hours after the stress ceases (Dhabhar et al., 1995).

Dhabhar has provided the clearest demonstration that temporary immunoredistribution associated with stress is triggered by corticosteroids (Dhabhar, 1998; Dhabhar and McEwen, 1996, 1997; Dhabhar et al., 1994, 1995, 1996), but the phenomenon of immunoredistribution has been recognized as an important trade-off response for at least the past 25 years. Fauci (1975) had already shown that corticosteroid treatment in guinea pigs leads to a temporary lowering of lymphocytes in peripheral circulation as they migrate to bone marrow. Chung et al. (1986) further showed that glucocorticosteroids in mice cause lymphocytes to migrate to peripheral lymph nodes and bone marrow. In fact, the temporary redistribution of various leukocytes in response to hormone stimulation is well documented (Claman, 1972; Cohen, 1972; Dhabhar, 1998; Dhabhar and McEwen, 1996, 1997; Dhabhar et al., 1994, 1995, 1996; Fauci and Dale, 1974; Gross, 1990; Landmann et al., 1984; Lundin and Hedman, 1978; Moorhead and Claman, 1972; Schedlowski et al., 1993; Spry, 1972) and can even be traced back to Dougherty and White (1944).

#### The testosterone immunoredistribution hypothesis

We propose, testosterone has a similar effect on the immune system to corticosteroids (i.e., immunoredistribution rather than suppression). Not only would suppression of the whole immune system be wasteful and maladaptive, but redistribution of immune cells would be an extremely valuable adaptive trade-off (Dhabhar, 1998). Just as emotional stress is often a good indicator of imminent trauma and immunochallenge, the testosterone surge in a displaying and competing male is also a good predictor of potential injury and immunochallenge. Male-male competitions are often not orderly, ritualized battles that avoid actual fighting. Rather, males in a wide range of species are often seriously injured, even killed, in competition over mates (Clutton-Brock, 1982; Cox, 1981; Geist, 1966, 1974; Silverman and Dunbar, 1980; Wilkinson and

Shank, 1977). Moreover, courtship interactions are always likely to involve some danger and stress because of the unpredictability of a potential mate's response (Hinde 1953, 1954). Therefore, we should expect immune resources to be temporarily redeployed to sites of potential injury.

We envision three possible mediating mechanisms for testosterone-mediated immunoredistribution. First, testosterone may directly activate immunoredistribution by binding to receptors in leukocytes or endothelium, thereby triggering migration to specific tissues (Figure 1a). Such receptors for steroid hormones have already been identified (Cupps and Fauci, 1982; Fox, 1995; Dhabhar, 1998). Second, testosterone may enhance corticosteroid levels which, in turn, trigger immunoredistribution (Figure 1b). Ketterson et al. (1991) and Ketterson and Nolan (1992) have demonstrated that experimentally elevated testosterone in dark-eyed juncos causes elevated corticosterone levels. Johnsen (1998) also found that seasonal elevations of corticosteroids and testosterone were significantly correlated. Finally, high testosterone levels may merely correlate with high corticosteroid levels due to the stress associated with male-male competition or courtship (Figure 1c). For example, the changes in circulating leukocyte frequencies in high-testosterone red jungle fowl males reported by Zuk et al. (1995) are well-known responses to stress and corticosteroids in domestic chickens (Gross and Siegel, 1983, 1985).

Although there is evidence that stress and cortisol inhibit testosterone under some situations (Rivier and Vale, 1984), testosterone levels do correlate with corticosteroid levels under more relevant circumstances, such as in the presence of reproductive females (McDonald et al., 1986; Silverin, 1998; Taylor et al., 1987). In addition, Sapolsky (1986) suggests that corticosteroids have different effects on testosterone level depending on a male's status in the group.

Our model contradicts what many believe to be settled science: that testosterone is immunosuppressive. The concept of immunosuppression by testosterone has been broadly accepted in the behavioral ecology literature and can be traced back to a number of reviews by Grossman (1984, 1985) and Alexander and Stimson (1988). These reviews have been repeatedly cited as the authority for the phenomenon of immunosuppression by testosterone (Folstad and Karter, 1992; Gal-

eotti et al., 1997; Hillgarth and Wingfield, 1997; Ros et al., 1997; Saino and Moller, 1994; Saino et al., 1995; Wedekind and Folstad, 1994; Zuk, 1994; Zuk and McKean, 1996). However, Grossman (1984, 1985) and Alexander and Stimson (1988) discuss the varying effects of steroids on different components of the immune system and are careful not to imply that all elements of immunity are stimulated or suppressed. They also review evidence for enhancement of different measures of immunity by both androgens and estrogens. The lack of strong evidence for immunosuppression by testosterone was recently noted by Hilgarth and Wingfield (1997), but they concluded that this points to a need for more research, rather than a need to question whether immunosuppression exists.

The reason for originally suspecting that testosterone is immunosuppressive appears to have come from the epidemiological finding that males suffer higher rates of infection and disease than females. However, Zuk (1990) pointed out that many incidences of disease and mortality in the males of many species are due to indirect effects of testosterone on behavior. For example, males often engage in riskier behaviors and are thereby exposed to different infectious agents than females. Hence, Zuk and McKean (1996) distinguish ecological from physiological causes in the sex differences in infection. In addition, we suggest that the sex differences in the rates of certain diseases may be due to the action of testosterone if redistribution leaves respiratory, digestive, or other systems less protected from infection. For example, Deerenberg et al. (1997) found that there was a lower immune response to an antigenic agent injected into the abdominal cavities of zebra finches during work or brooding stress. Similar reduced protection of the abdominal and thoracic organs due to immunoredistribution is also likely to result from increased testosterone. However, this would be a secondary effect of testosterone and not immunosuppression.

#### Mounting evidence against testosterone immunosuppression

There is a wide array of published data on enhancement of immunity correlated with testosterone. For example, Dunlap and Schall (1995) found that uninfected male fence lizards had unexpectedly higher levels of testosterone than infected males; Zuk et al. (1995) found that monocytes, heterophils and eosinophils all increased with increasing testosterone in red jungle fowl. Klein and Nelson (1998) found that testosterone correlated with higher specific immune response in voles. Ros et al. (1997) found that testosterone-treated male gulls had increased antibody titers. Although these are confounding results for any immunosuppression model, they are consistent with an immune system that temporarily redistributes its immune resources.

#### Predictions and tests

The suppression and redistribution models offer different explanations for phenomena such as reduction in circulating leukocytes and a wide range of different predictions follows from each hypothesis (Table 1). On the other hand, we share the prediction of the suppression model in expecting a priori to find similar effects of chronic and acute elevation of testosterone. Our expectation follows from the idea that testosterone would be a reliable predictor of potential trauma, whether acute or chronic. However, the immune response to chronic and acute stress may differ (Dhabhar, 1998; Dhabhar and McEwen, 1997). Therefore, if the effect of testosterone on immunoredistribution is mediated by corticosteroids (Figure 1b) or is correlated with stress (Figure 1c), there may be differences in the response to chronic and acute elevations of testosterone.

**Table 1**

#### Predictions of the testosterone immunosuppression and the testosterone immunoredistribution models

Type of response to elevated testosterone	Immuno-suppression	Immuno-redistribution
Leukocyte counts in blood and tissue	Decrease	No change in total number of leukocytes: quickly reversible decrease in source tissue(s) is accounted for by increase in sink tissue(s)
Response to acute elevation in T (e.g., during territorial display, mating display, or competition)	Decrease in number and activity of leukocytes	No change in total number of leukocytes: quickly reversible decrease in source tissue(s) is accounted for by increase in sink tissue(s)
Response to chronic elevated T (e.g., mating seasonal elevation)	Persistent decrease in numbers and activity of leukocytes	Redistribution may revert to baseline after an initial period if the mechanism of immunoredistribution is modulated by corticosteroids
Dermal response	Decrease	Increase because skin is an expected sink for redistribution to help reduce the risk of infection in the event of injury
Rate of respiratory, digestive, and parasitic infection	Increase due to suppression of immunity throughout the body	Increase because redistribution may result in a trade-off of reduced protection of some systems while enhancing protection against infection at wounds
Infection of wounds	Increase	Decrease
Activity of leukocytes	Decrease	Increase

To determine whether acute changes in testosterone levels result in males suffering from immunosuppression or benefiting from temporary immunoredistribution, one must time sample the indices of immune response. Dhabhar et al. (1995) found that a variety of leukocytes returned to baseline levels 3 h after stress treatments ended. Therefore, it would be crucial to test whether testosterone and circulating leukocytes experience parallel changes over time in displaying and competing males.

More specifically, we expect that some leukocytes are differentially locating to potential sources of injury and that peripheral immunity should increase with testosterone. Resistance to cutaneous infection should increase with testosterone, perhaps by the migration of macrophages and neutrophils which would best enhance defense against bacterial infection of a wound. Thus, we expect an enhanced dermal response in high-testosterone males similar to that found in stressed individuals (Dhabhar and McEwen, 1996).

Although the redistribution hypothesis specifically predicts changes in circulating leukocyte populations, it is founded on a general expectation that suppression is extremely maladaptive and should not be a common phenomenon (outside of normal negative feedback regulation of immunostimulation). Consequently, we would also expect enhancement of antibody-mediated immunity in response to elevated testosterone



as has been found in response to stress (Dhabhar and McEwen, 1996).

We look forward to testing these predictions experimentally in the immediate future. Until then, we believe that this hypothesis will offer a useful framework for interpreting the apparently confounding data collected by others who are currently examining the interactions between testosterone, immunity, and sexual selection.

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