Relative abundance of males to females affects behaviour, condition and immune function in a captive population of dark-eyed juncos *Junco hyemalis*

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Relative numbers of males and females in breeding groups may vary from expected values owing to a variety of factors. To determine how sex ratio might influence individual phenotypes in a captive population of dark-eyed juncos *Junco hyemalis* during the breeding season, we established three treatment groups: a male-biased (2:1), equal (1:1), and female-biased group (1:2). Within-group density (birds/m^2) was constant across groups. We assessed the frequency of flight chases (a proxy for social instability), measured changes in body mass and pectoral muscle condition, assayed plasma levels of testosterone (T) and compared cell-mediated immunity of individuals. We found significantly more chases in the male-biased group than in the female-biased group. Birds in the male-biased group lost more mass and displayed poorer pectoral-muscle condition than birds in the equal group. Cell-mediated immune responses were reduced in individuals in the male-biased group in comparison to the female-biased group. Plasma T levels in both sexes did not vary with sex ratio. Collectively, these results suggest that during the breeding season, social instability is greater in male-biased populations, and instability may lead to decreased general health and vigour.

In many migratory bird species, males arrive before females on the breeding grounds (Ketterson and Nolan 1976, Gauthreaux 1982). Until pairs have formed, social instability is high, and levels of the steroid hormones corticosterone (CORT) and testosterone (T) are elevated (Wingfield et al. 1990, Ketterson and Nolan 1992, Deviche et al. 2000). In monogamous species, the breeding sex ratio approaches 1:1 (Nolan et al. 2002), and as the breeding season progresses, both CORT and T levels decline (Wingfield et al. 1990, Ketterson and Nolan 1992, Deviche et al. 2000). Little is known about consequences of sex ratios that deviate from 1:1 throughout the breeding season.

Deviations from expected distributions of the sexes (normal breeding sex ratios) may arise. For example, cross-seasonal interactions, where events during the non-breeding season affect breeding season ratios (Marra et al. 1998), differential predation resulting from sex-typical differences in risky signalling behaviour, or sex-biased immigration to or emigration from breeding populations (Kempenaers 1994, Schradin and Lamprecht 2000, Croft et al. 2003), may lead to variation in local sex ratios. If social instability continues and results in a male-biased sex ratio, intense competition for mates and essential energy resources may arise and normal physiological processes may be altered.

Our goal was to explore the possible consequences of unbalanced sex ratios during the breeding season on physiology and behaviour in a monogamous songbird. To this end, we established three captive groups of dark-eyed juncos *Junco hyemalis* that varied in the abundance of males but were constant in terms of numbers of females, and bird density. In the first group, males outnumbered females 2:1, in the second group, males and females were equal at 1:1, and in the third group, females outnumbered males 2:1.

We predicted that when controlling for density, as the number of males increased in relation to a fixed

number of females, the number of competitive social interactions (e.g. aerial chases) would also increase, reflecting social instability. As a consequence of this social instability, we predicted that physiological processes (e.g. corticosterone or testosterone secretion) might be altered, leading to decreases in physical condition and immune function.

Methods and materials

Housing and care of birds

We housed 52 juncos (28 male and 24 female) near Bloomington, IN, USA (39°09′N, 86°23′W) in Indiana University's outdoor aviary. The aviary consists of 30 compartments (2.44 m × 2.44 m × 2.44 m), laid out in two parallel series (see Fig. 1). We grouped compartments into 3 sections, each section to be occupied by one of the three treatment groups of birds. Sizes of sections were varied corresponding to the inequality of size of treatment groups (details below). Birds were fed millet and sunflower hearts *ad libitum*; a protein-rich mixture of dog food, carrot, and hard-boiled eggs; and ~20 mealworms and 3 orange slices per compartment each week. Water was available *ad libitum* and changed three times a week; over a 4-week cycle, we treated water for three weeks with vitamins (Nekton-S, Pfprzheim, Germany), followed by one week with an antibiotic (sulmet), then three weeks of vitamins, etc.

Treatment of individuals; manipulation of male abundance

While female numbers were constant across groups (8 individuals), male numbers varied (16, 8, 4); therefore sex ratio also varied (2:1, male biased; 1:1, equal; 1:2, female biased). One individual from each group died during the experiment, changing group size and sex ratios to 16:7 (ratio 2.3:1), 8:7 (1:0.9), and 3:8 (1:2.4) at experiment end.

To compensate for the inequality of group sized we approximately equalized the mean areas of feeding space per bird across groups. More specifically, we allocated 14 compartments to the male biased group (4.09 m²/bird), 9 to the equal group (4.28 m²/bird) and 7 to the female-biased group (4.71 m²/bird; see Fig. 1).

Social interaction

To estimate the social stability of groups we counted numbers of flight chases during 10-minute observation periods in randomly chosen compartments in each treatment; all members of a group could move freely through all compartments of their section. Twelve observation sessions were conducted for each treatment before 10:00 on 4 days (10, 13, 18 and 19 June 2002). A chase was any instance in which one bird approached another in the focal compartment, induced it to fly, and then followed it rapidly and closely in the air to another perch or out of the observed compartment. The observer (TJG) was situated outside the compartment being observed (~3.7 m away), and the observer's presence did not cause noticeable disruptions in the compartment being observed. Movements of the birds were too rapid to distinguish identity or, in most cases sex.

Mass and condition

We measured mass and pectoral muscle condition of all birds at the beginning (31 May 2002), and conclusion of the study (10 July 2002). Changes in mass and condition were calculated for each treatment group. Mass was measured to the nearest 0.1 g (Pesola balance), and pectoral muscular condition was scored on Gosler's (1991) scale of 0 (highly convex) to 3 (highly concave).

Testosterone

We bled females during the mornings on 12–13 June 2002, and males on 27–28 June 2002. We held them (in some cases >1 h) in a dark bucket until we took ~200 μl of blood (in heparinized microcapillary tubes) from the alar vein. We then centrifuged the blood and collected, froze and stored the plasma until assayed.

Testosterone (T) concentrations were assessed using a commercial enzyme immunoassay (EIA) kit (#901-065; Assay Designs Inc., Ann Arbor, MI) following procedures described elsewhere by Clotfelter et al. (2004). The kit has a low (7.2%) cross reactivity with androstenedione and negligible (<1%) cross reactivities with dihydroepiandrosterone, oestradiol, dihydrotestosterone, progesterone, and corticosterone. The EIA kit manufacturer's procedures were followed exactly, with one exception: we used a 7-point standard curve that ranged in concentration from 200 pg/well to 3.125
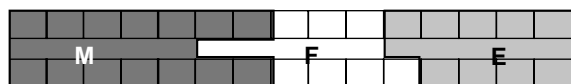

Fig. 1. Aviary: the aviary was divided into three sectors; each housed a free-flying group of birds. The number of females was kept constant among sectors and the number of males was varied. Bird density was kept constant by dividing the aviary compartments into the design presented above, where the male-biased group (M) had 14 compartments, the female-biased group (F) had 7 compartments and the equal group (E) had 9 compartments.

pg/well instead of a 5-point standard curve ranging in concentration from 200 (pg)/well to 0.781 pg/well. Testosterone concentrations were determined using a 4 parameter logistic curve-fitting program (Microplate Manager; Bio-Rad Laboratories, Inc., Hercules, CA) corrected for incomplete recovery. The intra-assay coefficient of variation was less than 5.76%, and the inter-assay coefficient of variation was 10.90%.

Cell-mediated immunity

We tested the effect of social environment on cell-mediated immunity by using a delayed-type hypersensitivity test, in which we measured wing-web swelling in response to an innocuous foreign plant protein, phytohemaglutinin (PHA; Sigma L-8754) (procedures modified from Lochmiller et al. 1993, Casto et al. 2001, with simplifications suggested by Smits et al. 1999). We primed birds (27–28 June 2002) with a subcutaneous injection of 50 μl of a solution of PHA in phosphate-buffered saline (PBS) (0.25 mg PHA in 50 μl PBS) into the scapular apterium of the left wing. Thirteen days after priming we obtained a baseline measure (to the nearest 0.01mm) on the left wing web. We used a pressure-sensitive digimatic thickness gauge (Mitutoyo 547–500) and then performed a challenge injection of 50μl of PHA solution into the left wing web. Twenty-four h thereafter we re-measured the wing-web to determine post-challenge change in thickness.

Statistics

Our purpose was to examine the effects of three sex ratios in all members of each of 3 groups. We therefore combined each group's males and females for statistical test. Two-sample T-tests revealed no significant sex differences between treatment effects (P's > 0.05). Because sample sizes, were small and the normality assumption was not met, we used non-parametric statistics to identify treatment effects (Kruskal-Wallis) in mass, pectoral muscle condition and flight chases (each observation session considered one data point). We probed significant main effects using pair-wise comparisons (Mann Whitney U-test) to assess differences between groups. A one-way ANOVA tested the effect of treatment on wing-web swelling, and post-hoc analysis (Tukey HSD) probed differences between groups. One-way ANOVAs assessed effects of treatment on T concentrations, and a T-test was used to determine sex differences in T concentrations with treatments combined. Effects were considered significantly different when P < 0.05.

Results

Conspecific interaction

The number of social interactions leading to flight chases differed among groups (H = 6.66, df = 2, P = 0.04) with higher frequency in the male-biased group as compared to the female-biased group (U = 29.0, P = 0.01), but no difference between the male-biased group and the equal group (P > 0.05) or between the female biased and the equal group (P > 0.05; Fig. 2).

Mass and condition

Seasonal change in body mass differed among treatments (H = 12.29, df = 2, P = 0.002; Table 1). The birds in the male-biased group lost significantly more mass than birds in the equal group (U = 48.5, P = 0.001), and tended to lose more mass than birds in the female-biased group (U = 62.5, P = 0.052). Changes in mass for the female biased and equal group did not differ significantly (P > 0.05; Fig. 3A).

Season-long changes in pectoral muscle condition were also affected significantly by treatment (H = 9.67, df = 2, P = 0.008; Table 1). Pair-wise comparisons showed significantly greater loss of condition in the male-biased group than in both the female biased (U =

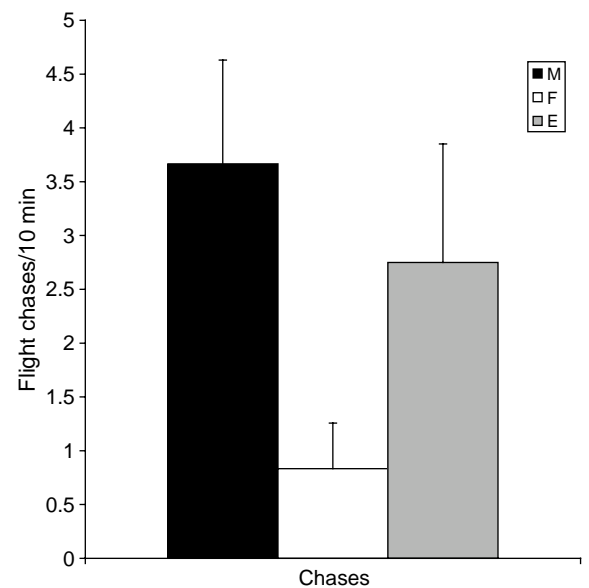Fig. 2. Treatment differences in frequency of flight chases (mean ± SE). Chases were more frequent in the male-biased (M) than the female-biased (F) group (P = 0.01). No differences in chase were detected between the male-biased (M) and equal (E), or between the equal (E) and female-biased (F) group.

Table 1. Initial and final body mass and pectoral muscle condition scores ($\pm$ SE).

	Body mass		Pectoral condition	
	Initial	Final	Initial	Final
Male biased group	21.56 ($\pm$0.28)	20.30 ($\pm$0.32)	2.0 ($\pm$0.00)	1.78 ($\pm$0.06)
Female biased group	21.78 ($\pm$0.50)	21.21 ($\pm$0.32)	2.0 ($\pm$0.00)	2.09 ($\pm$0.09)
Equal group	21.14 ($\pm$0.45)	21.24 ($\pm$0.28)	2.0 ($\pm$0.00)	2.13 ($\pm$0.08)

59.5, P = 0.017), and the equal group (U = 61.5, P = 0.004). No significant difference was found between the female biased and the equal group (P > 0.05; Fig. 3B).

Testosterone

Treatment had no detectable effect on male or female testosterone concentrations (P > 0.05 for both sexes; Table 2). Time from capture to blood sample did not predict T levels in males or females (males: R = 0.05, P > 0.05; females: R = 0.12, P > 0.05). When treatments were combined, females had lower concentrations of plasma testosterone than males (t = 16.687, df = 48, P < 0.0001).

Cell-mediated immune response

There was a significant effect of treatment on wing-web swelling (F = 6.69, df = 2, 42, P = 0.003). Birds in the male biased group displayed significantly less swelling than birds in the female biased group (P = 0.003), but the male-biased group did not differ from the equal group (P > 0.05), and the female biased and equal group did not differ from one another (P > 0.05; Fig. 4).

Discussion

We found that birds in the male-biased group displayed more social interactions, as well as decreased indicators of condition, including a loss in body-mass and pectoral muscle profile, and decreased cell-mediated immune responses to a challenge. Plasma levels of T, however, did not differ among treatments. Collectively, these results suggest that deviations in the abundance of males from expected values (1:1) can affect the behaviour and physiology of birds residing in a male-biased breeding population.

Local or regional breeding populations of migratory bird species are often composed of individuals that did not winter together, but rather, assembled from different portions of the species' non-breeding range. When conditions met on the winter range vary geographically, this variation may impact the breeding population (Marra et al. 1998), potentially altering breeding population sex ratios. The abundance of one sex may also vary due to differential mortality or because of sex-biased immigration to or emigration from breeding grounds (Kempenaers 1994, Schradin and Lamprecht 2000, Croft et al. 2003).

Our observation of more frequent social interactions in the male-biased group is consistent with our hypothesis that an increase in males throughout the
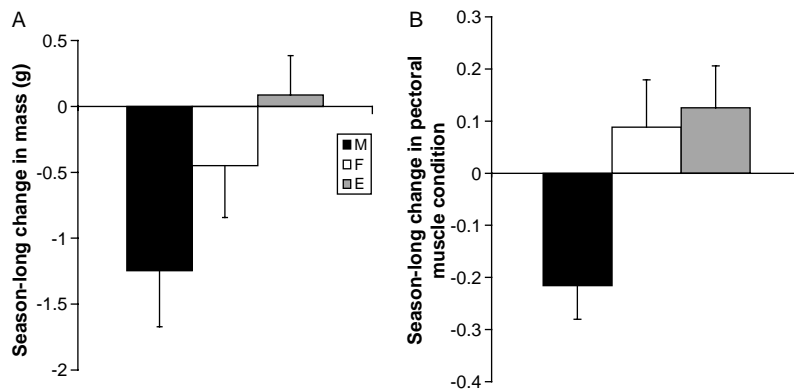

Fig. 3. Season-long change in body mass and pectoral muscle condition (mean $\pm$ SE): A) Juncos in the male-biased group (M) lost significantly more mass than juncos in the equal group (E; P = 0.002) and tended to lose more mass than juncos in the female-biased group (F; P = 0.052). B) Juncos in the male-biased group (M) lost condition, while juncos in the female-biased (F) and equal group (E) increased their condition scores. Condition change was significantly different between the male-biased group (M) and both the female-biased (F; P = 0.017) and the equal group (E; P = 0.004), and did not differ between the female-biased (F) and equal groups (E).

Table 2. Mean testosterone values of males and females in each treatment ($\pm$ SE).

	Male-biased group	Female-biased group	Equal group
Male	3.32 ng/ml (± 0.10)	3.53 ng/ml (± 0.08)	3.12 ng/ml (± 0.12)
Female	1.47 ng/ml (± 0.14)	1.32 ng/ml (± 0.04)	1.55 ng/ml (± 0.21)

breeding season would lead to social instability in a local breeding population. Reductions in immune function, body mass and pectoral condition in the male-biased group are also consistent with the view that, for a given population density, social upheaval is higher when males outnumber females. Due to constraints on catching and bleeding the birds rapidly after initial disturbance, we were unable to relate plasma levels of corticosterone, a steroid hormone associated with stress, with treatment. It has previously been demonstrated, however, that corticosterone levels in males are elevated prior to breeding, a time associated with territorial establishment and aggression when female densities are low (Deviche et. al 2000). Similarly, in comparison to individually housed animals, when captive female or male Siberian hamsters cohabit with conspecific males, circulating cortisol levels are elevated and acquired immune responses are reduced (Demas et al. 2004). Several other studies have also shown that chronic social and environmental stress can be immunosuppressive (Zalcman et al. 1991, Dhabahar and McEwen 1996, Sheridan 1998, Carobrez et al. 2002, Endo et al. 2002, Ruiz et al. 2002).

The loss in mass and decrease in pectoral (flight) muscle condition in the male-biased group are likely results of increases in the social upheaval/environmental stress levels. Behaviourally, these reductions might be attributable to increased competition for resources and

inhibition of feeding behaviours associated with stress (Carr 2002). Although unable to assess in our study, chronic exposure to elevated CORT is known to produce flight muscle atrophy in captive juncos (Gray et al. 1990, Casto unpubl. obs.). Whatever the mechanism underlying reductions in mass and condition, the current study may underestimate the influence of a sex ratio biased towards males on body mass and muscle condition under free-living conditions in which there is no *ad libitum* access to high quality food.

Plasma T did not vary with treatment. One possible explanation for the failure to detect an effect of sex bias on testosterone is that we sampled males relatively late in the study, after behavioural observations of social instability were recorded. T levels in the dark-eyed junco, and many other songbirds, are elevated at the beginning of the season, but fall to basal levels as breeding begins (Ketterson and Nolan 1992, Wingfield 1994), and the same may be true in captive juncos regardless of male abundance. PHA immune responsiveness was measured at the same time as T samples in males, and a reduction in PHA wing-web swelling response was found in the male-biased group. This suggests that physiological variables other than T (e.g. energy balance or response to elevated CORT) may be driving the observed socially-induced immunosuppression.

In sum, surprisingly little is known about the physiological and behavioural consequences of changes in social structure/stability due to alterations in sex ratios. While reproductive opportunities are predicted to improve for the rarer sex (Fisher 1930), there may also be potential costs (i.e. losses in pectoral condition and immune responsiveness) of membership in a male-biased population that need to be taken into account and could likely influence population dynamics. Furthermore, if a female benefits from settling on a territory with other females, such as increased survivorship owing to enhanced immune functioning and greater energetic stores, then these benefits may be large enough to overcome a polygyny threshold (Verner and Willson 1966), in turn potentially contributing to variation in mating systems on a local scale. Although the design and the number of individuals in our study precluded us from investigating sex differences, future studies should investigate how differences in the abundance of one sex affects the physiology and behaviour of the opposite sex.
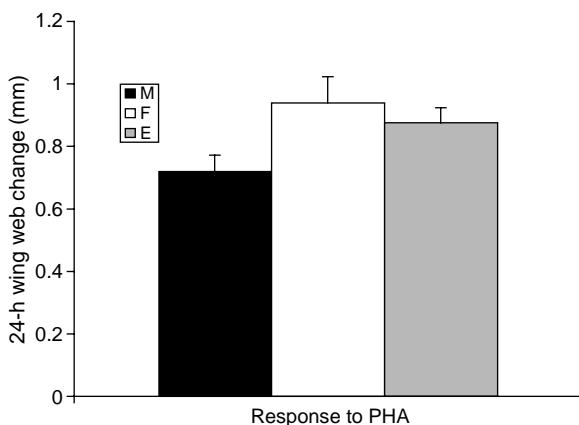

Fig. 4. Treatment difference in immune response to PHA challenge (mean $\pm$ SE): swelling was less pronounced in the male-biased (M) than in the female-biased group (F; P = 0.003). Swelling in the equal group (E) did not differ from the male-biased (M) or female-biased group (F).

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