Molecular sexing of prey remains permits a test of sex-biased predation in a wintering population of western sandpipers

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Recd 20.10.03; Accptd 26.01.04; Published online 09.03.04

Population sex ratios in monogamous birds are often male biased. One factor that can affect population sex ratios is sex-biased predation. However, most estimates of sex-biased predation in birds have focused on species with obvious sexual colour dimorphism or body size dimorphism. Data on sexually monomorphic birds are generally lacking. In the present study, we adopt a PCR-based sexing procedure to help test for sex-biased predation in a wintering population of western sandpipers (*Calidris mauri*), a shorebird that shows only subtle sexual size dimorphism. Specifically, by comparing the *a priori* determined sex ratio of live birds wintering at a site in western Mexico to the molecular estimate obtained from depredated birds at this same site, we were able to perform a population-specific test for sex bias in predator-induced mortality. The proportion of females estimated from living (ca. 25%) versus dead (ca. 24%) individuals was in fact not significantly different, indicating that the strong male bias in this population is not due to differential predation. However, molecular sexing of prey remains is a hitherto unexploited test of sex-biased predation in birds, and is potentially applicable to any species for which prey remains can be gathered. We discuss our results in the context of alternate ecological hypotheses for population sex biases.

Keywords: sex ratio; western sandpiper; *Calidris mauri*; predation; molecular sex determination; non-breeding distribution

1. INTRODUCTION

Population sex ratios in birds often diverge from parity and are, in fact, predominantly male biased (Trivers 1971; Bellrose 1980; Dobson 1987; Breitwisch 1989; Sargeant & Raveling 1992). While recent studies have shown that some birds are able to manipulate primary offspring sex ratios (e.g. Komdeur *et al.* 1997; Sheldon 1998; Green 2002), most species tend to lay an equal proportion of males and females (Clutton-Brock 1986; Breitwisch 1989). In these species, any observed bias in sex ratio must be due to subsequent sex-biased mortality. Factors

promoting sex-biased mortality are not necessarily due to predation, but may instead be caused by other sex-based differences in life history, such as feeding specialization (Durell & Goss-Custard 1996) or parental investment strategies (Trivers 1971; Owens & Bennett 1994). By contrast, a population sex bias could be directly associated with predation if, for example, morphological, physiological or behavioural differences rendered one sex significantly easier to detect or capture (Angelstam 1984; Widén *et al.* 1987; Götmark 1993; Slagsvold *et al.* 1995; Götmark *et al.* 1997).

Although the need for better estimates of sex-specific mortality has been voiced (e.g. Promislow *et al.* 1992), such estimates remain uncommon, especially those that can clearly identify the cause of the sex bias in mortality. Studies identifying predation as the cause for sex-biased mortality are usually restricted to species showing obvious sexual dimorphism, which permits the direct sexing of prey remains, or they focus on birds during the breeding season (Widén *et al.* 1987; Götmark 1993; Slagsvold *et al.* 1995; Götmark *et al.* 1997). New approaches to measuring differential predation are therefore desirable and, accordingly, we have adopted a novel approach that relies in part on the PCR-based sexing procedure of Griffiths *et al.* (1998), and is potentially applicable to any species for which prey remains can be gathered. In the present study, we demonstrate the application of this approach for, and conduct a test for sex-biased predation in, a wintering population of western sandpipers (*Calidris mauri*).

Western sandpipers are small migratory shorebirds. Males and females show no differences in plumage coloration (Wilson 1994), but females are slightly larger than males and have longer bills (Cartar 1984). Western sandpipers breed in Alaska and eastern Siberia and spend the non-breeding season mainly along the American Pacific coast between southern Canada and Peru (Wilson 1994; Nebel *et al.* 2002). Males and females are not distributed evenly across the non-breeding range, however, as females tend to migrate further south than males, resulting in a latitudinal bias in population sex ratio (Nebel *et al.* 2002). Whether differential predation contributes to local sex biases in birds has never been tested.

2. STUDY AREA AND METHODS

Data were collected between January and March 2000 at Bahía Santa Maria, Sinaloa, Mexico (24.0° N, 108.0° W), one of the major non-breeding sites of western sandpipers (Morrison *et al.* 2001). This Pacific estuary covers an area of ca. 1350 km² and is composed of mangroves, intertidal and brackish mudflats and freshwater marshes (Engilis *et al.* 1998). Population sex ratios in this northern part of the non-breeding range are biased towards males, and indeed, a mist-netted sample of 823 individuals collected during this study period contained only 25% females (Nebel *et al.* 2002). The main predators of western sandpipers at this site are peregrines (*Falco peregrinus*) and merlins (*F. columbarius*), and these predators have been shown to impose a significant mortality cost on other populations of wintering shorebirds (Page & Whitacre 1975; Whitfield 1985). In fact, prey remains, such as part of a wing, were commonly observed at this site, typically near plucking posts or close to the high-water mark on the mudflats (S. Nebel, personal observation). For the purpose of this study, birds whose carcasses were found dismembered and eviscerated were considered 'predated', whereas carcasses not clearly showing these characteristics were not and consequently were not included in our analysis. While it is possible that individuals could have been dismembered or eviscerated by scavengers following death by other means, this seems unlikely to us because scavengers were never observed near our study area.

To determine the sex ratio of recently depredated individuals, we first removed 5–15 primary feathers from their carcasses. Feathers were then frozen, and transported back to the laboratory for DNA analysis. Genomic DNA was extracted from feather shafts using a

DNeasy Tissue Kit (Qiagen). The *CHZ* and, in the case of females, the *CHW* loci were PCR amplified following the procedure of Griffiths *et al.* (1998), using the primers P2 (5'-TCTGCATCGCTAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRAAYTG-3'). PCR products were then separated by electrophoresis on a 3% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. As expected, females, being the heterogametic sex, were readily identified by the presence of two bands (*ca.* 360 and *ca.* 380 bp), whereas males were identified by the presence of a single (*ca.* 360 bp) band. Finally, the frequency distribution of males and females among living versus dead groups was compared via a 2 × 2 contingency table.

3. RESULTS

We were able to determine the sex of 25 depredated individuals from their remains via molecular criteria. Out of these, six individuals (24%) were found to be female, 18 individuals male. The frequency distribution of females and males among living birds at this site was found to be 25% and 75%, respectively (Nebel *et al.* 2002). Therefore, the proportion of females and males among depredated versus living birds was not statistically different (Pearson $\chi^2 = 0.017$, d.f. = 1, $p = 0.90$), indicating an absence of sex bias in predation. A lack of evidence for differential predation is unlikely to be a consequence of the relatively small number of depredated birds that we were able to sample. An observation of less than three (or greater than 10) females would have been sufficient to detect differential predation, given our sample size ($p < 0.05$, power = 0.80).

4. DISCUSSION

Population sex biases in avian species are common (Bellrose 1980; Dobson 1987; Breitwisch 1989; Sargeant & Raveling 1992), but the factors driving these biases are not well understood. Predation is one of the factors that could lead to a population sex bias, however, estimates of sex-biased predation are difficult to obtain, particularly for species with no obvious morphological sexual dimorphism. We demonstrated that molecular sexing of prey remains could be used to test for sex biases in predation. This approach, especially if applied widely and to multiple populations, could potentially reveal new and large-scale patterns of sex-biased predation in birds, or, alternatively, falsify the idea that sex-biased predation is at all important.

Based on studies conducted to date, population sex ratios in waterfowl and songbirds appear to be male biased (Bellrose 1980; Breitwisch 1989; Sargeant & Raveling 1992). While these patterns are usually attributed to differential parental investment, where females bear higher costs producing eggs or rearing young (Breitwisch 1989; Sargeant & Raveling 1992), empirical evidence supporting this assumption is mostly absent (but see Owens & Bennett 1994). In western sandpipers, this explanation is unlikely to account for the male bias at northern wintering sites because, unlike songbirds, it is males that do most of the chick rearing, as females depart several weeks before males from the breeding grounds (Wilson 1994).

Alternatively, a male bias in local sex ratio at wintering sites of western sandpipers may stem, at least in part, from differential predation. This hypothesis is motivated by the fact that female western sandpipers have a larger body size than males (Cartar 1984), and from this we generally expect females to have proportionally higher wing loading

(mass/wing area) (Pennycuik 1972) and a concomitant decrease in escape performance (Norberg 1995; Burns & Ydenberg 2002). Quick take-off from the ground is crucial in escaping predators (Cresswell 1993) and wing loading might therefore lead to increased predator-induced mortality. This hypothesis has, however, never been tested and, to our knowledge, this study provides the first empirical test for sex-biased predator-induced mortality in a nearly monomorphic bird at a non-breeding site.

The sex ratio of western sandpipers at Bahía Santa Maria, Sinaloa, Mexico, did not differ between live birds in the population and those killed by predators. However, while female western sandpipers are generally larger than males and are known to have higher wing loading at three other non-breeding sites, no difference in wing loading has since been detected at our study site, despite a consistent difference in body size (Nebel 2003). The apparent absence of wing loading differences at our study site may therefore explain the absence of differential predation observed in the present study. Differential predation, if it occurs in western sandpipers, might therefore be a population-specific phenomenon and not a ubiquitous feature of the species.

Future work, adopting the approach described here and expanding its application to include, for example, multiple populations covering large geographical areas, will help build on the preliminary findings reported here and further document the presence and/or extent of sex-biased predation in birds.

Acknowledgements

Guillermo Fernández facilitated fieldwork in Mexico, Virgilio Antonio Pérez helped with data collection, and Gilberto Salomon, Patolandia Hunting Club, Mexico, provided logistical support for this study. Dov Lank, Theunis Piersma and Ron Ydenberg provided valuable comments on the manuscript.

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