GENERAL INTRODUCTION

Reproduction is the result of male and female interaction. Although both sexes have to cooperate to pass on their genes to next generations and to increase their share of a species' gene pool, they differ in strategies to optimize their reproductive output. Traditionally conflict between the sexes has been viewed to arise because males produce many small gametes (sperm) whereas females produce few large gametes (eggs) (Andersson 1994). Theoretically males can repeatedly mate with many females while females are limited in their opportunities to reproduce and their fitness can be significantly lowered by inappropriate matings (Trivers 1972). Because commonly females are the sex, which bears the greater cost of reproduction and reproductive error, they represent the limiting factor for reproduction. Therefore male-male competition over access to females (intrasexual selection) and female choice (intersexual selection) originates (Andersson 1994).

Sexual selection defined by Darwin (1871) as "selection that arises from differences in mating success" at first has been seen to occur exclusively "before parents unite" (Darwin 1871) and subsequent studies maintained this precopulatory emphasis (Andersson 1994). In a pioneering paper Parker (1970) called attention to the fact that male contest over reproduction may continue after insemination. In promiscuous species males compete via their ejaculates for fertilization of a given set of ova, a phenomenon described as ‘sperm competition’. Postcopulatory male-male competition has subsequently been observed to ubiquitously occur from plants to mammals (Birkhead and Møller 1998).

It took nearly 25 more years until also the idea of cryptic female choice extended the possibility of female choice past initiation of copulation (Eberhard 1996). Female controlled processes and structures enable females to selectively influence paternity of males with particular traits even after insemination. In particular the potential female ability to select sperm within her reproductive tract in order to increase her fitness became a focus of scientific discussion (Birkhead 1998; Birkhead 2000; Eberhard 2000; Kempenaers et al. 2000; Pitnick and Brown 2000). However, so far the evidence for its existence is ambiguous (e.g. insects: Wilson et al. 1997, Clark et al. 1999, Stockley 1999, Mack et al. 2002, Nilsson et al. 2003, Bretman et al. 2004; fish: Evans et al. 2003, Pilastro et al. 2004; lizards: Olsson et al. 1996; birds: Birkhead et al. 2004; but see Stockley 1997, Birkhead et al. 1999, Cunningham and Cheng 1999).

An extreme case of sexual conflict in the narrow sense (enhancement of reproductive success of one sex at the expense of fitness of the other sex (Parker 1979)) may occur in species where males are able to force females to copulate. This behavior is found in a variety of species (primates: Smuts and Smuts 1993; birds: McKinney et al. 1983; reptiles: Olsson 1995; fish: Farr 1980; insects: Thornhill 1980). Despite extreme behavioral resistance, females often appear to have no control over their copulatory partners. Cryptic female choice may than be a mechanism, which evolved to circumvent precopulatory male manipulation and to assure female reproductive optimum.

This thesis investigates male and female influences on paternity and focuses on evaluation of cryptic female sperm selection as a mechanism shaping male reproductive success. For this purpose we chose wild mallards, *Anas platyrhynchos*. In this duck species forced copulations represent a major component of the mating system (McKinney et al. 1978; McKinney et al. 1983), although mallards form socially monogamous pair bonds in autumn (Cramp 1977). Females show strong preferences for their social partner (Cunningham 2003) and offspring viability as well as mother productivity is significantly reduced when females are paired with non-preferred males (Bluhm and Gowaty 2004). However the possession of an intromittent organ facilitates forced copulations by extra-pair males (McKinney and Evarts 1997), which are always heavily resisted by the female (McKinney and Evarts 1997; Cunningham 2003). Nonetheless these extra-pair copulations can result in fertilization (Burns et al. 1980; Evarts and Williams 1987).

The main aims of this thesis were to investigate

1. *Evidence for cryptic female choice in wild mallard populations*
   We examine levels of extra-pair paternity in wild mallard populations and compared it to reported frequencies of extra-pair copulations. (Chapter One)
2. *Relative importance of cryptic female sperm selection and sperm competition in determining male fertilization success*
   By inseminating groups of related females with a sperm mixture containing equal number of sperm from one brother and from one unrelated male in combination with measurement of sperm quality, we

compare postcopulatory male and female influences on paternity. (Chapter Two)

3. *Potential mechanism of female influence on sperm competition*

   Sperm swimming speed is an important determinant in sperm competition. We look at differential female influence on this male trait. (Chapter Three)

Further aspects of male and female reproductive decisions were investigated during this study.

Male side to reproduction:

1. *Extra-pair paternity*

   Forced extra-pair copulations are well-documented in waterfowl. We report on the extra-pair fertilization success of this male reproductive strategy in wild mallard populations. (Chapter One)
2. *Trading copulation frequency against ejaculate quality*

   Production of ejaculates is a timely and costly process. We examine the costs of frequent copulations in terms of reduced competitiveness of single ejaculates. (Chapter Four)
3. *Testosterone and testis size*

   Testis size is correlated with sperm production in many species. We investigate if it is also related to production of the sex steroid testosterone, which is an important factor governing female mate choice and male reproductive behavior in mallards. (Chapter Five)

Female side to reproduction:

1. *Manipulation of primary sex ratios*

   If each sex provides different fitness benefits, gender of offspring will be an important determinant of female reproductive success. We determine primary sex ratios in wild mallards in general and in consecutive nesting attempts. (Chapter One)

2. *Brood parasitism*

   By laying eggs in foreign nests ducks can significantly increase their reproductive output. We quantify levels of brood parasitism in two wild mallard populations and relate it to nesting density as a potential constraining factor of egg dumping. (Chapter One)

Finally the appendix provides technical information on the microsatellites used for paternity and maternity assessment in this study.

CHAPTER ONE

GENETIC ANALYSIS OF SEX RATIOS, BROOD PARASITISM AND EXTRA-PAIR PATERNITY IN MALLARDS (*ANAS PLATYRHYNCHOS* L.)

Angelika G. Denk & Bart Kempenaers

ABSTRACT

Mallards, *Anas platyrhynchos*, are among the most common waterfowl species in the Northern Hemisphere. Despite their abundance and despite growing interest of behavioral ecologists and evolutionary biologists in key aspects of their behavior, few studies have used genetic tools to investigate their mating system. We studied the breeding biology of mallards by examining 41 clutches from two areas that differ in breeding density. We focus on three aspects of mallard reproductive behavior. First, adult sex ratios in mallards are often reported to be male-biased. In our population, the proportion of males observed during autumn and winter counts varied between 59% and 67%. Here we show that this bias is already present in the primary sex ratio: on average 60% of eggs in a clutch are males. Second, intra-specific brood parasitism is observed in many duck species. We found egg dumping in 53% of mallard clutches in a high breeding density area, whereas brood parasitism was entirely absent in an area with low breeding density. Third, although mallards are socially monogamous, forced extra-pair copulations are frequently observed. Using microsatellites, we estimate that a minimum of 56% of broods contained at least one extra-pair young.

Overall, at least 14% of fertilized eggs were sired by an extra-pair male. Breeding density did not influence the proportion of broods with extra-pair paternity. However, broods from the high density area contained significantly more extra-pair young than broods from the low density area.

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INTRODUCTION

Sexual selection is an important evolutionary agent shaping morphology, mating behavior and life history (Andersson 1994). Individuals face strong selection to pursue reproductive strategies that ultimately increase their fitness. Waterfowl, in particular ducks (*Anatinae*), exhibit three notable features of their reproductive system, which should be closely linked to reproductive success: male-biased sex ratios at the population level, egg dumping by female ducks and forced extra-pair copulations. Here, we report on a study of free-living mallards (*Anas platyrhynchos*), one of the most common waterfowl species in the Northern Hemisphere (Cramp 1977). We use molecular tools to investigate primary sex ratios as well as the occurrence of intra-specific brood parasitism and extra-pair paternity. The aim of our study is to provide a detailed description of the mating system of mallards and to discuss the adaptive value of individual reproductive decisions.

Sex ratios

Sex ratio manipulation is a classic example of an individual's reproductive decision that can be explained by evolutionary theory (Sheldon 1998). If daughters and sons provide different fitness benefits to their parents, selection should favor parents which produce the more profitable sex (Charnov 1982). A paradoxical situation arises in waterfowl populations, which are frequently observed to be male-biased (Bellrose et al. 1961; Gowaty 1993; Blums and Mednis 1996 and citations therein). Adult sex ratios of two to three or more males per female are recorded for canvasbacks (*Aythya valisineria*) and common pochard (*Aythya ferina*) (Rohwer and Anderson 1988; Oring and Sayler 1992). Also in mallards adult sex ratios with significant skews in favor of males are frequently reported, and it is unlikely that this can be attributed to pure sampling errors (see Fig. 1).

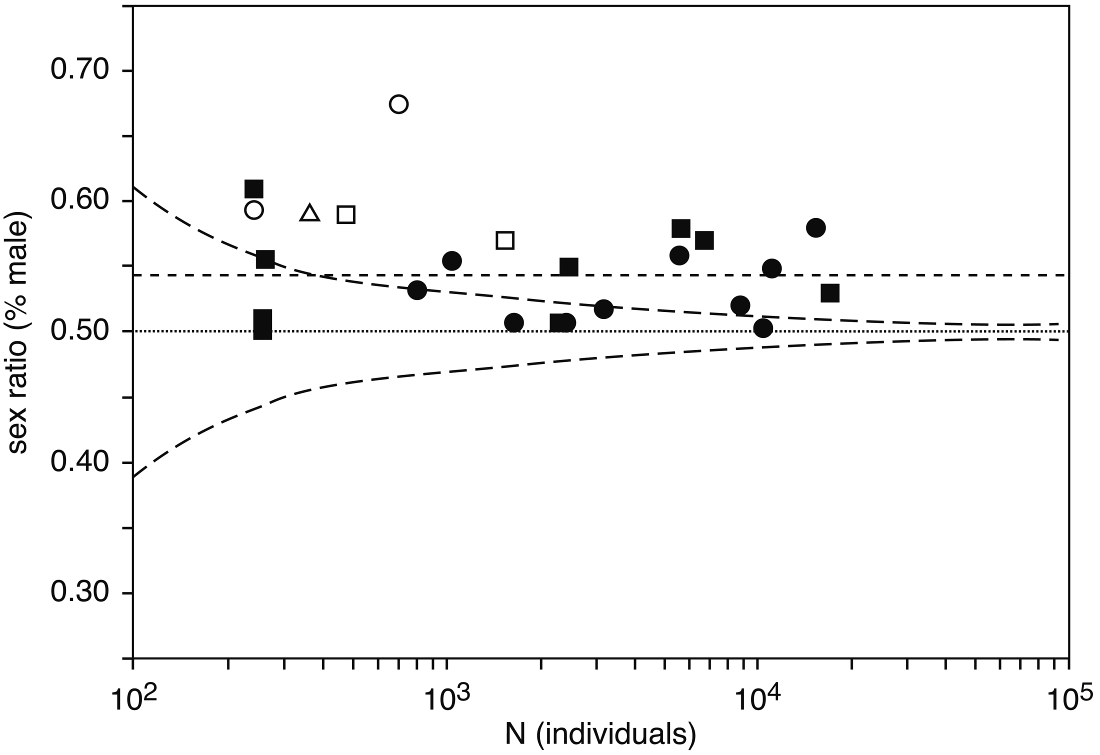

Figure 1. Population sex ratios of wild mallards. Data (filled symbols) are obtained from field observations reported in (Bellrose et al. 1961; Humburg et al. 1978 and Ferguson et al. 1981); filled circles: all data; filled squares: data restricted to counts in March and April; counts obtained from trapping and hunting are excluded due to potential bias). Open symbols show adult sex ratios observed in this study at Lake Starnberg and Lake Ammer (circles: October count; squares: January count). The triangle indicates the primary sex ratio (this study). The dotted line indicates the null hypothesis of parity; curved, long-dashed lines display binomial significance