CHAPTER 7

Male Seychelles Warblers face a trade-off between mate guarding and extra-pair fertilizations

Cas Eikenaar
David S. Richardson
Jan Komdeur

Submitted manuscript

Abstract

Mate guarding may cause a trade-off between the benefits of protecting paternity and the benefits of competing behaviours, such as pursuing extra-pair fertilisations (EPFs). Studies showing a temporal separation of mate guarding and extra-pair copulations (EPCs) support this view. However, no studies have shown direct evidence for a trade-off between mate guarding and EPFs. In this study, we investigate the timing of EPFs in the Seychelles warble, a species in which males intensively guard their mate during the fertile period. Our results show that males often gained EPFs before or after, but seldom during the fertile period of their female, indicating a trade-off between mate guarding and gaining EPFs. In such a system, if breeding synchrony is high, opportunities for males to pursue EPFs would be few. We would, therefore, expect the occurrence of EPFs to be negatively correlated with local breeding synchrony. We found no such correlation in the Seychelles Warbler. This may be because the combination of relatively low breeding synchrony and high breeding density observed in this species results in there always being plenty of non-guarding males available to attempt EPFs. .

Introduction

In most socially monogamous bird species extra-pair fertilizations (EPFs) occur frequently (Griffith *et al.* 2002) and result in decreased reproductive success for the cuckolded male. To minimize this cost, male birds have evolved a variety of elaborate anti-cuckoldry tactics of which mate guarding is the most common (Birkhead *et al.* 1987; Birkhead and Möller 1992). By following his female closely during her fertile period the male attempts to prevent her from copulating with other males (Beecher and Beecher 1979; Birkhead 1979). Mate guarding has been shown to decrease the number of extra-pair copulations (EPCs) of the guarded female (e.g. Björklund & Westman 1983; Birkhead *et al.* 1989; Kempenaers *et al.* 1995; Komdeur *et al.* 1999) and decrease the level of cuckoldry (Currie *et al.* 1998; Chuang-Dobbs *et al.* 2001). Intensive mate guarding may, however, also impose costs on the male and may cause a trade-off between the benefits of protecting paternity and the benefits of competing actions, such as attracting additional mates or seeking EPCs (Westneat *et al.* 1990; Chuang-Dobbs *et al.* 2001). If a male guards his female, this will reduce the number of opportunities he has to engage in EPCs, especially when the reproductive cycles of the females within the population are synchronised (Birkhead and Biggins 1987; Westneat *et al.* 1990).

Several studies have shown that during mate guarding there is a reduction in male behaviours associated with EPCs (Buitron 1983; Brodsky 1988; Westneat 1988; Currie *et al.* 1998), or the frequency with which EPCs were gained by males (e.g. Aguilera & Alvarez 1989; Hunter *et al.*1992). However, EPCs do not necessarily result in EPFs (Gyllensten *et al.* 1990; Hunter *et al.* 1992; Dunn *et al.* 1999; Török *et al.* 2003). Michl *et al.* (2002) and Fossøy *et al.* (2006) experimentally prevented the social mate from fertilizing his social partner, and showed that practically all females do engage in EPC, but that the natural rates of EPY production are much lower. Hence, EPC behaviour need not result in the production of EPY. For example, all female Bluethroats, Luscinia s. svecica, are promiscuous and often have high proportions of EPY in their broods, but about half of them do not have any (Fossøy *et al.* 2006).

In order to determine if mate guarding constrains a male's ability to gain EPFs, the study system has to fulfil three requirements: (i) the fathers of all extra-pair young must be accurately identified, (ii) the fertile periods of the pair and the extra-pair female need to be determined, and (iii) the level of opportunities for EPFs, i.e. the number of available receptive females – which will be influenced by breeding synchrony - should be known (Birkhead & Biggins 1987; Kempenaers 1997; Stutchbury 1998).

The Cousin Island population of the Seychelles Warbler (*Acrocephalus sechellensis*), a rare endemic of the Seychelles islands, has been the focus of intensive study since 1985 (e.g. Komdeur 1992; Komdeur 2003; Richardson *et al.* 2001; Richardson *et al.* 2003a,b). Primary males intensively follow the primary female during her fertile period (Komdeur *et al.* 1999; Komdeur 2001) and mate guarding ends immediately after

the single egg has been laid (Komdeur *et al.* 1999). Mate guarding in the Seychelles Warbler is paternity guarding; if males spent more time mate guarding they are less often cuckolded (Komdeur *et al.* 2007) EPCs are male driven; females were never seen outside their own territory during their fertile period and territory intrusions were always performed by males (Komdeur *et al.* 1999). EPFs are always sired by a primary male from another territory as opposed to subordinate males from other territories (Richardson *et al.* 2001). Given the high risk of cuckoldry in these warblers (40% EPF; Richardson *et al.* 2001), they are a suitable species to study the trade-off between mate guarding and EPFs.

In this study we attempt to answer two questions. First, do male Seychelles Warblers face a trade-off between mate guarding and obtaining EPFs? Second, if males trade mate guarding with seeking EPFs, does this result in a decreased risk of being cuckolded when the level of breeding synchrony is high (Birkhead and Biggins 1987; Westneat *et al.* 1990)?

Methods

Study Population and Data Collection

The present study is based on data gathered on Cousin Island during the main breeding periods (June-September) in 1997–1999. During this time almost all birds (> 96%, Richardson *et al.* 2001) were individually colour-ringed and blood-sampled, and all territories (105–112 per year) on the island were mapped in detail. Nests were systematically searched for by following the resident primary female for 30 minutes (Komdeur 1992), at least once every two weeks. Once located, nests were observed throughout the breeding cycle. When nest-building was nearly finished, nests were checked daily for eggs. Blood samples (ca 15 μl) were collected from adults and 10–16 day old nestlings by brachial venipuncture and then stored in 800 μl of 100% ethanol at ambient temperature. Adult birds were trapped using mistnets and nestlings were taken out of the nest to be sampled. After sampling, birds were released or put back into the nest immediately and unharmed. It is very unlikely that catching or sampling has a negative long-term effect on the warblers; annual adult survival probability is astonishingly high (89%; Brouwer *et al.* 2006). Permission to study, catch, ring and take bloodsamples of Seychelles Warblers was granted by the Seychelles Ministry of Environment and Natural Resources.

Seychelles Warblers usually produce only a single egg each breeding season (91%, n = 223, Komdeur 1996). The length of the peak fertile period of female Seychelles Warblers is assumed to span from day -6 (where day 0 is the day of egg laying) until the day before the single egg is laid (see also Birkhead and Möller 1992; Sundberg 1992; Pagenkopf and Wesolowski 2002). We chose this time period, because in the Seychelles Warbler mate guarding started six days before egg laying and was absent from the day of egg laying onwards (Komdeur *et al.* 1999), and all copulation at-

tempts were observed in this time period (Komdeur *et al.* 1999). Furthermore, studies on other passerines that have examined when copulations actually result in fertilization, suggest that the duration of the peak fertile period is probably not more than six days (Smith *et al.* 1996; Lifjeld *et al.* 1997; Sheldon and Ellegren 1998).

Parentage

DNA extraction, genotyping and parentage analysis was completed using CERVUS (Marshall *et al.* 1998) and the methodology described in Richardson (2001) as part of another study (Richardson *et al.* 2003a). The simulation program within CERVUS was used to estimate the required critical differences in LOD (the natural logarithm of the likelihood ratio) scores between the first and second most likely candidate parent. Parentage was analysed for all offspring sampled in 1997-1999, but only offspring which were blood-sampled in the nest (and for which we have an accurate egg lay date) were used in the present study. For this study the minimum level of confidence in the paternity assignment was set at 75%.

Status Definitions

The status of the birds involved in this study was defined as follows. The extra-pair young (EPY) is fathered by the extra-pair male. The extra-pair male lives on a different territory from the EPY and is mated on that territory to the extra-pair female. The EPY grows up in the nest of the pair female and the pair male. The pair male is the male that is cuckolded.

Data Analyses

For each EPF, the difference in days between the lay date of the pair and extra-pair female was calculated by subtracting the latter from the former. In passerines, eggs are fertilized approximately 24 h before they are laid (Howarth 1974; Birkhead and Möller 1992). Therefore, assuming a six day fertile period, if the difference in lay date of the pair and the extra-pair female fell within the range –5 to +5 days, the EPC that resulted in the EPF could have been gained during the period in which the extra-pair male was mate guarding (figure 7.1). If this difference fell outside the –5 to +5 range, then the EPF was most likely gained before or after the period during which the extra-pair male was mate guarding.

In the Seychelles Warbler most EPFs are gained very close to the resident territory; in our sample 70% (23 out of 33) of EPFs were gained one or two territories away from the extra-pair male's own territory. Since there were between 105 and 112 territories on the island each year and a radius of two territories holds about 13 territories, EPF appears to be a local phenomenon in this species. Therefore, the calculation of the expected number of EPFs gained was done on a local scale. The exact date of egg laying of both the pair and extra-pair female was known for 23 EPFs that were gained in neighbouring territories (≤ 2 territories between the pair and extra-pair males' territories). For each of the 23 females producing an EPY (the pair females),

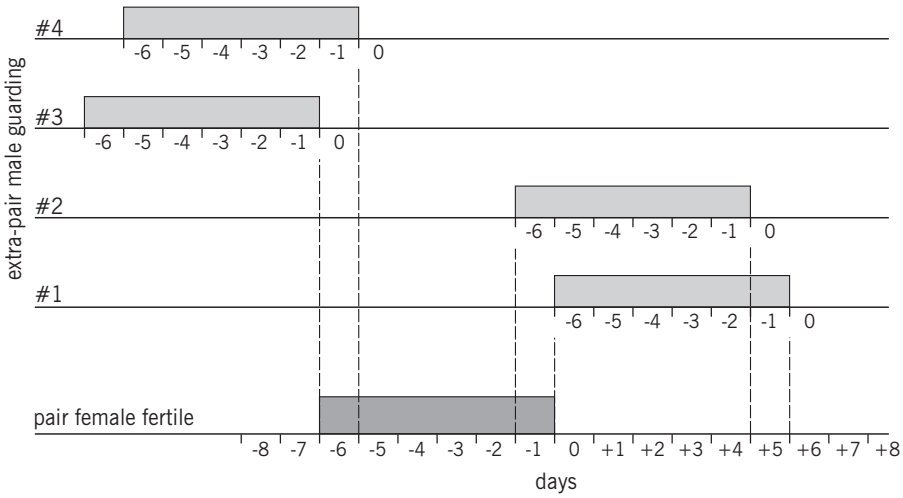

Figure 7.1. The fertile period of a pair female (lower graph) and the guarding period of four hypothetical extra-pair males whose extra-pair females laid their eggs, 6 days after (#1), 5 days after (#2), 6 days before (#3) and 5 days before (#4) the pair female. Day 0 represents the day of egg laying. The figure shows that if the difference in lay date of the pair and the extra-pair female fell within the range -5 to +5 days, the EPC that resulted in the EPF could have been gained during the extra-pair male's period of mate guarding.

the difference (in days) between their laying dates and those of all the females present in neighbouring territories were calculated by subtracting the latter from the former. For each pair female, the proportion of these differences lying between –5 and +5 days was calculated. This proportion gives the expected probability that the fertile period of the pair female and the guarding period of the extra-pair male overlapped if mate guarding does not affect a male's ability to pursue EPCs. These probabilities were summed to give the expected number out of the 23 EPFs for which the fertile period of the pair female and the guarding period of the extra-pair male overlapped (and hence also by subtraction the expected number where these periods did not overlap). Using Chi-square tests, these two expected frequencies were then compared with the observed frequencies. In our sample the first EPF was gained 19 days before, and the last EPF 21 days after the extra-pair female laid her egg. Although opportunities for EPFs existed before and after this period, males did not appear to use these, probably because they were not sexually active at that time. This idea is supported by the fact that cloacal protuberances (indicative of sperm storage) of male Seychelles Warblers remained small until about 20 days before the pair female laid her egg and decreased in size dramatically about 20 days after egg laying (Van de Crommenacker *et al.* 2004). The data set therefore included only neighbouring females that laid between 19 days before and 21 days after the female producing the EPY, thus encompassing the sexually active period of the males.

To assess whether the trade-off between mate guarding and EPFs affected the risk of being cuckolded, we compared the local breeding synchrony of nests that contained an EPY, sired by a male from a neighbouring territory (≤ 2 territories away), with nests that did not contain an EPY. Every clutch of one egg between 1997–1999 for which paternity was known was used. We used two measures of local breeding synchrony. First, synchrony was calculated as the number of females in neighbouring territories with fertile periods not overlapping with that of the focal female (Johnson and Lifjeld 2003). Second, an index value of breeding synchrony, taking the duration of fertile period overlap into account, was calculated following Kempenaers (1993). Index values are the average proportion of females in neighbouring territories that were fertile on each day during the fertile period of the focal female.

To test whether local breeding synchrony was different between nests with and without (local) EPFs, for both measures of local breeding synchrony a separate binary logistic regression was performed using SPSS 11.5 (2001). Whether or not an EPF occurred was entered as the dependent variable, synchrony was entered as a covariate and year as a categorical covariate. Using the backward stepwise method non-significant variables were removed from the model. Probability values are two-tailed and the null hypothesis was rejected at $p < 0.05$.

Results

Trade-off between mate guarding and EPFs

The X-axis in fgure 7.2A shows how many days apart the 23 females producing an EPY (the pair females) and all their neighbours (≤ 2 territories away) laid their egg. The Y-axis in this figure shows how often each of these differences in lay-dates occurred. The zero on the X-axis represents complete synchrony between the pair female and a neighbouring female. A negative value means that the pair female laid before, and a positive value means that the pair female laid after a neighbouring female. Figure 7.2B is similar to Figure 7.2A, but shows how many days apart the pair females and their extra-pair counterparts laid their egg. In this figure, the –5 to +5 days period on the X-axis is the period in which males could have gained their EPF during the fertile period of their own female, i.e. during their mate guarding period. Figure 2b shows that the mate guarding period of the extra-pair male and the fertile period of the pair female usually did not overlap, i.e. very few (13%; 3 of 23) EPFs were gained during the period of mate guarding. The expected number of EPFs (based on figure 7.2A) gained during mate guarding was 7.44 EPFs, which was significantly higher than the observed number of EPFs (Chi-square test: $n = 23$, $\chi^2_1 = 3.92$, $P < 0.05$).

Local breeding synchrony and EPFs

A male's risk of losing paternity through EPFs did not depend on the number of neighbouring males that were not mate guarding at the time this male's female was

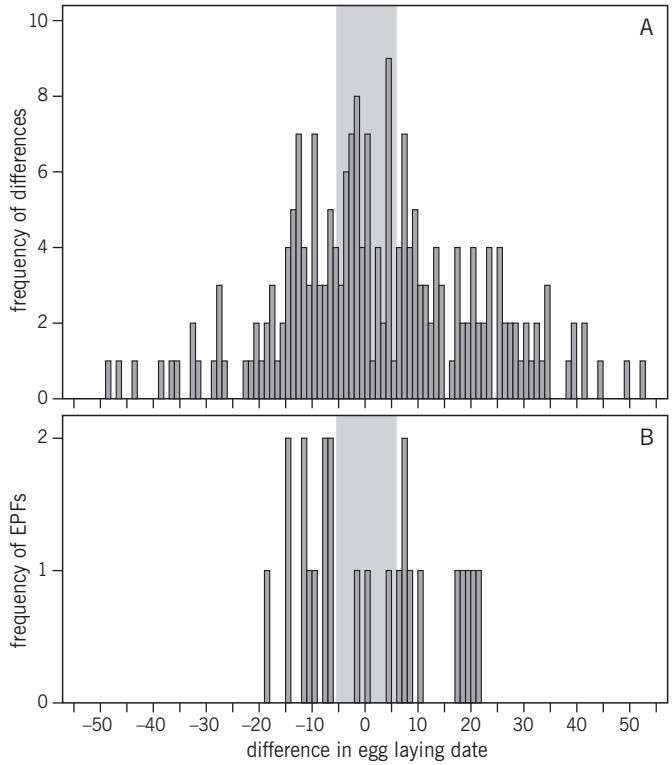

Figure 7.2. The frequency distributions of the difference (in days) between the laying dates of the 23 females producing an EPY (the pair females) and A) those of all their neighbours (≤ 2 territories away) and B) the 23 extra-pair females. The zero on the X-axis represents complete synchrony between the pair female and, in A), a neighbouring female or, in B), the extra-pair female. A negative value means that the pair female laid before, in A), a neighbouring female or, in B), the extra-pair female. A positive value means that the pair female laid after, in A), a neighbouring female or, in B), the extra-pair female. The shaded area indicates the –5 to +5 days period, the period in which the EPC that resulted in the EPF could have been gained during the mate guarding period of the extra-pair male.

fertile; local breeding synchrony did not affect the frequency of EPFs (figure 7.3). The number of females in neighbouring territories with fertile periods not overlapping with the fertile period of the focal female was not different between nests that did or did not contain an EPF (Binary logistic regression: $N_1 = 51$, $N_2 = 23$, *Wald* = 0.03, d.f. = 1 and $P = 0.87$). Neither was the synchrony index value different between nests that did or did not contain an EPF (Binary logistic regression: $N_1 = 51$, $N_2 = 23$, *Wald* = 1.28, d.f. = 1 and $P = 0.26$). The interaction between year and synchrony was not significant in both models ($P > 0.51$). The mean local synchrony value was 6.83 % ($n = 74$).

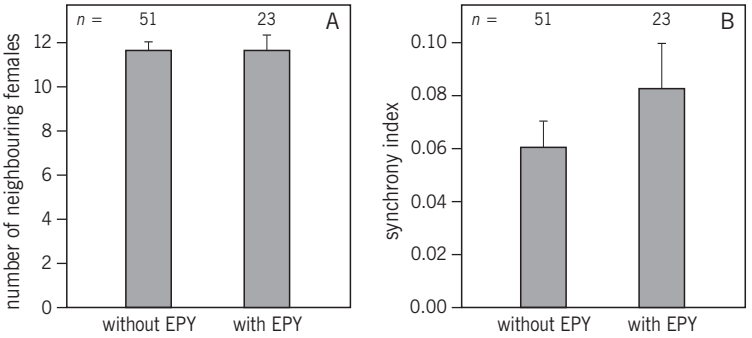

Figure 7.3. A) The number of females in neighbouring territories with fertile periods not overlapping with that of the focal female (i.e. with lay date more than five days earlier or later than egg laying in the focal nest) of nests without and with an EPY. B) Local breeding synchrony indices of nests without and with an EPY, sired by a male from a neighbouring territory (≤ 2 territories away). Index values indicate the average proportion of fertile females in neighbouring territories on a given day during the period when the focal female is fertile. Bars indicate means ± s.e..

Discussion

To our knowledge our study is the first to provide direct evidence that male birds face a trade-off between mate guarding and EPFs. Significantly fewer EPFs were gained during the guarding period of the extra-pair male than expected if mate guarding would not affect a male's ability to pursue EPCs. This indicates a temporal separation of mate guarding and seeking EPFs.

This finding is in agreement with most studies that investigated the timing of behaviours associated with EPCs (e.g. intrusions into other males' territories; Buitron 1983; Brodsky 1988; Westneat 1988; Hobson and Sealy 1989; Johnson and Kermot 1989; Currie *et al.* 1998) or timing of the actual EPC frequency (e.g. Frederick 1987; Aguilera & Alvarez 1989; Hunter *et al.*1992). In a few species, no trade-off between mate guarding and EPC behaviour was found (Afton 1985; Edinger 1988; Sorensen 1994; Kempenaers 1997; Johnson and Lifjeld 2003). In some of these species there is a rather large individual variation in the risk of being cuckolded (Sorensen 1994; Kempenaers 1997; Johnson and Lifjeld 2003). This variation may result from female choice of extra-pair mates (Hasselquist *et al.* 1995), such that high quality males can leave their mate unguarded without risking lost parentage (Stutchbury 1998), while low quality males do face a trade-off between guarding and EPCs (Kempenaers *et al.* 1995; Johnson and Lifjeld 2003). As a result, one group of males may invest little time in mate guarding and more in seeking EPFs during their mate's fertile period, while the other group of "less attractive" males follow a best-of-a-bad-job strategy by investing heavily in mate guarding (Kempenaers *et al.* 1995; Johnson *et al.* 1998). Moreover, in some of these species, females seem to have the upper hand in the sexu-

al conflict over fertilizations by actively pursuing EPCs on the extra-pair male's territory. Female Bluethroats seem to be able to escape male paternity guards (Johnson *et al.* 1998) while female Blue Tits, *Parus caruleus*, actively seek EPCs by visiting high quality males on their territories (Kempenaers *et al.* 1992). Therefore, the outcome of a potential trade-off between EPFs and mate guarding may be masked by the females' EPC behaviour in these species.

The extent to which males separate mate guarding and the pursuit of EPFs may, in conjunction with breeding synchrony, have an effect on the frequency of EPFs. If males trade-off EPC behaviour with mate guarding, and EPCs are predominantly male driven, higher breeding synchrony may result in lower levels of EPFs (Birkhead and Biggins 1987; Westneat *et al.* 1990). Alternatively, when females pursue EPFs and males do not face a trade-off between mate guarding and seeking EPFs, higher breeding synchrony could increase the level of extra-pair fertilisations, as females would be able to simultaneously compare displaying males and assess their relative quality (Stutchbury and Morton 1995). Seychelles Warbler males do appear to face a trade-off between mate guarding and EPCs are predominantly male driven. We therefore expected that the risk to be cuckolded would decrease with increasing local breeding synchrony. The reason that we did not find such a relationship may be that, although differences in local breeding synchrony were observed, synchrony values were generally low (figure 7.2A; mean local synchrony: 6.83%, whereas many species have synchrony values higher than 20% (Stutchbury 1998)). Furthermore, because breeding density of the studied population was high (105–112 territories on 29 ha.) non-guarding males may always be available in close proximity to the fertile female to attempt an EPF.

Acknowledgements: Nature Seychelles kindly allowed us to work on Cousin Island and provided accommodation and facilities during the stay. The Department of Environment and the Seychelles Bureau of Standards gave permission for fieldwork and sampling. We thank D. Bekkevold, K. Blaakmeer, T. Clarke, G. Davison, D. Dowling, J. Drachmann, C. Eising, M. Fowlie, I. Hartley, K. van Oers, and T. Veen for help in the field, D. Dawson, F. Jury and P. Salgueiro for help in the laboratory and Ellen Kalmbach, Kate Lessells and two anonymous referees for constructive comments on the manuscript. This work was supported by grants from the National Environmental Research Council to Terry Burke and Jan Komdeur (GR3/11154), the Large Australian Research Council (A19802459) and the Netherlands Organisation for Scientific Research (NWO-VICI,86503003), both allocated to Jan Komdeur. David Richardson was supported by a NERC fellow ship (NER/i/s/2002/00712) .