Adaptive value and development of nest-building in Red Bishops (Euplectes orix)

Von der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg zur Erlangung des Grades und Titels eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

angenommene Dissertation

von Herrn Markus Metz

geboren am 29.09.1971 in Friedrichshafen

Gutachter: Dr. Thomas W.P. Friedl

Zweitgutachter: Prof. Franz Bairlein

Tag der Disputation: 2. Mai 2005

Contents

An automated analysis of avian forebrain activity in relation to nest-building in Red Bishops (*Euplectes orix*) 91

General Introduction

1 Nests and nest-building in birds

Morphological and behavioural differences between the sexes in animals are often a result of sexual selection (Andersson 1984). In birds, conspicuous behaviours and traits like song and plumage colouration are well known to influence reproductive success through sexual selection, but the importance of nests and nest-building behaviour for reproductive success and as possible cues for mate choice received only little attention. The vast majority of birds build some sort of nest or at least choose a nest site to improve reproductive success. Nests and their location can function as protection against sun, rain, wind, heat, cold and predators (Hansell 2000). Usually the exclusive function of nests is to protect offspring. In most bird species, even if sexes differ in some other behavioural or morphological traits, both sexes together engage in nest-building, incubation and feeding of nestlings. Although a nest and raising offspring is crucial for offspring survival, it is difficult to determine the contribution of each sex to offspring survival with regard to nest-building, incubation and feeding of nestlings, if both sexes together engage in these activities. In many weaverbirds, males build nest frames without the participation of females (Skead 1956, Emlen 1957). These nest frames get accepted by females who line them and pad the inside with soft material. In these species, the contribution to offspring survival is clearly divided between the sexes: males alone build nest frames in their territory, whereas females incubate eggs and feed nestlings. In polygynous weaverbird species with the described division of labour between the sexes, male reproductive success might not only be determined by plumage characteristics or courtship behaviour, but also by the ability to build many and/or high-quality nests (Collias & Victoria 1978). Nests can influence male reproductive success in two ways. First, males with more nests built during a season can attract more females (Friedl & Klump 1999), second, females could choose males based on their nests and evaluate the quality of nests.

2 Objectives of this thesis

This PhD thesis was initiated by results of a previous study conducted by T. W. P. Friedl, who investigated various aspects of the breeding system of Red Bishops

(*Eulectes orix*; Friedl & Klump 1999, 2000, Friedl 2002, 2004). Red Bishops are a polygynous, colonially breeding weaverbird species widespread in sub-Saharan Africa. Males built nest frames which get accepted by females, whereas females incubate eggs and raise offspring without male assistance (Skead 1956), as is typical for polygynous weaverbird species, (Emlen 1957). Amongst other results, Friedl (1999) found that male reproductive success is mainly determined by the total number of nests build within a breeding season, which in turn depends on the duration of territory tenure and the number of nests built per week. The objective of the thesis presented here was to investigate various aspects of male nest-building behaviour and their influence on male reproductive success in more detail.

The first chapter analyses the influence of several aspects of nest-building ability and effort, nest characteristics, and male age on nest-building performance and reproductive success. A number of nest characteristics are tested for their influence on nest durability and matching acceptance probability. Changes in reproductive success, nest-building ability and effort with male age are investigated and discussed in light of different hypotheses (Forslund & Pärt 1995) explaining age-dependent reproductive success. In particular, the following questions are addressed in the first chapter: (1) How does male breeding performance depend on nest-building skills and effort? (2) Do females show a preference for certain nest characteristics, and if yes, do these nest characteristics influence nest durability or breeding success? (3) Do males of different age classes differ with respect to reproductive success, and if yes, are the observed differences due to reproductive effort, nest-building skills or the quality of nests built?

The second chapter deals with male time-budget allocations and nest-building efficiency in relation to nest-building performance and reproductive success. Additionally, a relation between selected behavioural patterns, especially male-male interactions, and territory tenure is tested for. Further on, a possible trade-off in the time budgets allocated to different behaviours is investigated. The following questions are addressed: (1) Do males that construct more nests during the breeding season spend more time with nest-building, and if so, are there any trade-offs with other behavioural patterns? (2) Do males that construct more nests during the breeding season build more efficiently than other males, indicating better inherent or acquired nest-building ability? (3) Independent of the number of nests built by a male, do other aspects of nest-building behaviour influence male reproductive success? (4)

In addition, I investigate whether other behavioural patterns such as the proportion of time spent with male-male interactions or courtship behaviour are related to male reproductive effort (number of nests built) or reproductive success (number of nests accepted).

The third chapter describes the Red Bishops' breeding system as a biological market with nests as a traded commodity following the considerations on biological markets by Noë & Hammerstein (1994, 1995). Temporal changes in demand for and supply of nests within a breeding season on a weekly time scale are described and their consequences for male nest-building behaviour are analysed. The timing and synchrony of male and female breeding activity and their interaction with each other and rainfall are described. Short-term adjustments in the supply of nests by males in response to increasing female demand for nests are investigated, together with an effect of such adjustments on male mating success.

The fourth chapter analyses forebrain activity in relation to nest-building behaviour. According to Jarvis & Mello (2000) it can be expected, that nuclei from all major forebrain subdivisions are involved in processing sensory and motor information of any complex behavioural pattern. Nest-building behaviour in weaverbirds is a complex sequential motor pattern and needs to be learned in the first two years of life (Collias & Collias 1964, 1973), similar to singing in passerines. It is assumed that neural control of nest-building is as complex as neural control of singing, also involving several different regions in the forebrain. Since this is the first study investigating forebrain activity in relation to nest-building behaviour in weaverbirds, all major forebrain subdivisions shall be analysed for changes in activity after nest-building. Forebrain activity is measured as protein expression of an Immediate Early Gene (IEG), ZENK. The investigation of brain area activity by the expression of IEGs is an established technique (Chaudhuri 1997). It can be applied in a variety of contexts (Long & Salbaum 1998), because the expression of IEGs is generally associated with contexts in some way important for the organism (Clayton 2000), e.g. novel contexts and memory formation. Regions involved in the control of nest-building behaviour are identified by changes in ZENK expression after nest-building. Additionally, differences between hemispheres in ZENK expression in relation to nest-building behaviour are investigated.

I hope that this thesis raises more interest in the importance of nests and nest-building behaviour, features that can determine reproductive success in addition to other behavioural and morphological traits. Furthermore, I suggest that the investigation of neural mechanisms underlying nest-building behaviour could lead to a better understanding of the functional organisation of the avian forebrain.

3 References

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ

Chaudhuri, A. 1997. Neural activity mapping with inducible transcription factors. Neuroreport 8(13): III-VII

Clayton, D.F. 2000. The genomic action potential. Neurobiology of Learning and Memory 74(3): 185-216

Collias, E.C., Collias, N.E. 1964. The development of nest-building behaviour in a weaverbird. Auk 81: 42-52

Collias, E.C., Collias, N.E. 1973. Further studies on development of nest-bulding behaviour in a weaverbird (*Ploceus cucullatus*). Animal Behaviour 21: 371-382

Collias, N.E., Victoria, J.K. 1978. Nest and mate selection in the Village Weaverbird (*Ploceus cucullatus*). Animal Behaviour 26: 470-479

Emlen, J.T.Jr. 1957. Display and mate selection in the whydahs and bishop birds. Ostrich 28: 202-214

Forslund, P., Pärt, T. 1995. Age and reproduction in birds – hypotheses and tests. Trends in Ecology and Evolution 10(9): 374-378

Friedl, T.W.P. 2002. The effect of rainfall on the breeding behaviour of the Red Bishop, *Euplectes orix*. Ostrich 73(3-4): 181-184

Friedl, T.W.P. 2004. Breeding behaviour of the Red Bishop (*Euplectes orix*): a synthesis and new observations. Die Vogelwarte 42: 177-190

Friedl, T.W.P., Klump, G.M. 1999. Determinants of male mating success in the red bishop (*Euplectes orix*). Behavioural Ecology and Sociobiology 46: 387-399

Friedl, T.W.P., Klump, G.M. 2000. Nest and mate choice in the red bishop (*Euplectes orix*): female settlement rules. Behavioral Ecology 11: 378-386

Hansell, M. 2000. Bird nests and construction behaviour. Cambridge University Press, Cambridge

Jarvis, E.D., Mello, C.V. 2000. Molecular mapping of brain areas involved in parrot vocal communication. Comp Neurol 419(1): 1-31

Long, K.D., Salbaum, M. 1998. Evolutionary conservation of the Immediate Early Gene ZENK. Mol Biol Evol 15(3): 284-292

Noë, R., Hammerstein, P. 1994. Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behavioural Ecology and Sociobiology 35: 1-11

Noë, R., Hammerstein, P. 1995. Biological Markets. Trends in Ecology and Evolution 10(8): 336-339

Skead, C.J. 1956. A study of the red bishop bird. Ostrich 27: 112-126

Zur Homepage der Dissertation

Chapter 1

The importance of male nest-building effort and ability, nests, and male age for reproductive success in the Red Bishop (*Euplectes orix*)

1 Abstract

In the highly polygynous and colonially breeding Red Bishop birds, male mating success is mainly determined by the number of nests a male builds during a breeding season. A detailed analysis of nest-building effort and skills revealed that higher male mating success independent of the number of nests built depends on nest-building skills in terms of a smaller proportion of unusable nests and a smaller proportion of abandoned nest-building attempts. The number of nests built, being the main determinant of male mating success, increased with longer territory tenure (more weeks a male held a territory), with a shorter time delay between finishing a nest and starting a new nest, and with fewer days needed to build a nest. Effects of male morphology on mating success and nest-building performance were not apparent. Nests were measured and characterised by the density of fibres in the breeding chamber and several parameters describing size and deformation. Females preferred nests that were more densely woven and nests that had a larger entrance roof overlap. The durability of not accepted nests increased with a larger entrance roof overlap, thus increasing the number of nests a male had available for females. The probability of breeding failure due to a nest becoming squashed while it contained eggs or nestlings was reduced when the breeding chamber was denser. Mating success in male Red Bishops differed between young and old males due to differences in the number of nests built with young males building fewer nests, which in turn was related to shorter territory tenure times and longer delays between building two consecutive nests in young males. No differences between age classes were found with regard to nest-building skills in terms of the proportion of abandoned nest-building attempts and the proportion of unusable nests.

2 Introduction

Individual reproductive success can be influenced by a wide variety of different factors. Such factors could enhance the ability to acquire a high number of mating partners in a polygamous mating system. By definition, individual reproductive success varies substantially in polygamous species and sexual selection pressure is much more pronounced than in monogamous species (Andersson 1994), enhancing the influence of certain traits on reproductive success. This pressure can be intersexual selection in the form of, usually, female choice or intrasexual selection. Intrasexual selection in birds can act in the form of direct male-male competition where males compete for access to females or resources like territories (e.g. Savalli 1994), or more indirectly through differences in male quality such as endurance rivalry (Andersson 1994) or nest-building performance (Friedl & Klump 1999).

A number of studies in this context focussed on effects of male morphological and behavioural traits such as size (e.g. Kruuk *et al.* 1999, Lindenfors *et al.* 2002), colouration (review in Hill 1999, Pryke *et al.* 2002) or song (review e.g. Catchpole & Slater 1995) on mating success. A similar importance for mating success through sexual selection is known for certain structures built by males. A widespread example for this function of such structures are nests or nest-like structures of some fish (e.g. Sikkel 1995, Ostlund-Nilson 2000, Svensson & Kvarnemo 2003) and bird species (e.g. Collias & Victoria 1978, Borgia 1985, Madden 2003). The original and most important function of nests is to protect offspring and to guarantee breeding success. In species where nests or certain parts of the nest are constructed by one sex only, mating success might depend on quantitative (e.g. Savalli 1994, Evans & Burn 1996) or qualitative (e.g. Hoi *et al.* 1994, Madden 2003) aspects of nest structures. Nest construction and characteristics in turn can be related to builder quality (e.g. Barber *et al.* 2001, Doucet & Montgomerie 2003).

The nests of weaverbirds (Passeriformes: Ploceidae) belong to the most elaborate nests found in birds in terms of structure and skills required. Furthermore, nests or at least initial nest frames are built by males only (Skead 1956, Emlen 1957). Therefore it seems likely that nests play a role in determining male mating success (Collias & Collias 1984). However, there are only few studies that investigated the effect of nest quantity or quality on mating success in weaverbirds. The number of nests has been shown to be positively related to male mating success in the Yellow-shouldered Widowbird *Euplectes macrourus* (Savalli 1994a), the Red-shouldered Widowbird *Euplectes axillaris* (Pryke & Andersson 2003) and the Red Bishop *Euplectes orix* (Friedl & Klump 1999, Lawes *et al.* 2002). In weaverbirds, effects of nest quality on mating success have only been investigated for the Village Weaver *Ploceus cucullatus*. Collias and Victoria (1978) showed in aviary studies that females preferred green (i.e. fresh) nests over brown (i.e. old) nests, but since males also displayed at higher rates at fresh nests (Collias & Victoria 1978) it remains unclear whether the observed female preference was based on nest characteristics or male behaviour. In the same species, external closeness of weave described by the frequency and degree of loose loops protruding from the surface of the nest did not affect female acceptance behaviour (Collias & Victoria 1978).

In Red Bishops, male mating success is mainly determined by the number of nests a male builds during a breeding season, which varies considerably among males (Friedl & Klump 1999, Lawes *et al.* 2002). Territories defended by male Red Bishops in the study colony do not contain food sources, only nest sites, as was found for other colonies of Red Bishops (Craig 1974). Male Red Bishops do not engage in parental care like incubation of eggs or feeding of nestlings. Thus the sole contribution of male Red Bishops to offspring is a nest and genetic material. This explains the observed high level of polygyny with one male achieving up to 20 matings in terms of accepted nests per season. From the female perspective, there seem to be no trade-offs in terms of shared contribution to paternal care when mating with a male that already has a mate. It is not known why certain male Red Bishops build so many more nests than other males. Possible factors can be the ability to defend territories for a longer time, to defend larger territories with more potential nest sites or the ability to build nests faster. These factors can be summarised as reproductive effort. Earlier studies have shown that the number of nests built correlated with territory tenure (Friedl & Klump 1999), territory size (Lawes *et al.* 2002), and the number of nests built per week (Friedl & Klump 1999).

Not only quantitative but also qualitative aspects of nest-building might influence male mating success in Red Bishops. Such qualitative aspects could be the proportion of usable nests built by a male, or the proportion of nest-building attempts that actually resulted in a complete nest and were not abandoned before being finished. These qualitative aspects allow assessment of sensory and motor skills required to build a nest and might represent builder quality. Nest size, density of weaving, or nest stability could all be used by females to assess the quality of a nest. By choosing nests based on certain nest characteristics, females might be able to gain either direct benefits, if certain nest characteristics are related to breeding success, or indirect benefits, if nest characteristics indicate male genetic quality, for example motor or learning skills.

Reproductive success varies with age in a number of bird species (e.g. Collis & Borgia 1993, Evans 1997, Espie *et al.* 2000, Penteriani *et al.* 2003). I investigated whether male mating success also varied with age in Red Bishops, and if these differences were related to experience in terms of better nest-building skills or to age-related reproductive effort. Additionally, I looked for differences in nest characteristics in relation to the age of the building male. There are three general explanations for age-dependent reproductive performance, first, progressive appearance or disappearance of phenotypes, second, improvements of experience and competence, and third, optimization of reproductive effort (review in Forslund & Pärt 1995). The results will be examined for which of the three explanations matches them best.

In summary, the following questions are addressed in this study: (1) how does male breeding performance depend on nest-building skills and effort, (2) do females show a preference for certain nest characteristics, and if yes, do these nest characteristics influence nest durability or breeding success (3) do males of different age classes differ with respect to reproductive success, reproductive effort, nest-building skills or the quality of nests built?

3 Methods

3.1 Study colony

I studied a colony of Red Bishops in the Addo Elephant National Park in South Africa in the Eastern Cape Province (33°26´ S, 25°45´ O) during three consecutive breeding seasons (2000/2001, 2001/2002 and 2002/2003). The colony was situated at a small pond. Nests are typically suspended in bulrushes (*Typha capensis*) or reeds (*Phragmites australis*). 50 males established a territory in the season 2000/2001, compared to 64 males in the season 2001/2002 and 55 males in the season 2002/2003. All territorial males were in the typical bright red and black breeding plumage. One year old males in non-breeding plumage also built nests and showed courtship behaviour, but these nests were not accepted by females.

3.2 General field methods

All nests of adult territorial males were individually marked with small tags attached to reeds close to the nest. Nests were controlled every other day to check for the state of the nest, number of eggs and nestlings in nests and to mark new nests. For each nest I recorded the date the male started building it (entrance ring detectable), the date of finishing (breeding chamber closed), the date of acceptance (occurrence of first egg), and the date on which it became unusable. A nest was regarded unusable when it was partially or completely destroyed, for example by Cape Weavers (*Ploceus capensis*) or Masked Weavers (*Ploceus velatus*) that often removed a Red Bishop nest to build their own nest in that place. A nest could also be rendered unusable when it was deformed to a degree that it was no longer possible for a bird to enter it because of a compressed entrance or an indented breeding chamber. Since most nests were accepted within two weeks after they were finished (see Chapter 3), the number of unusable nests comprised only nests that became unusable within two weeks after they were finished. Birds were caught using mist nets and traps baited with commercially available mixed seeds. Each bird was ringed with a numbered metal ring obtained from the South African Bird Ringing Unit and a unique combination of four coloured plastic rings for individual identification. Previous studies have shown that rings with colours similar to secondary sexual traits are able to influence male and female mating behaviour (e.g. Burley *et al.* 1982, Metz &

Weatherhead 1991, Johnson *et al.* 1993, Johnson *et al.* 1997). Therefore each individual was equipped with a red ring to avoid a possible influence of this ring on mating behaviour.

The identity of the male building a given nest was determined by its combination of coloured rings and, if a nest was accepted, I tried to identify by which female it was accepted, again through its combination of coloured rings.

As morphological features, wing length, tarsus length and weight of adult birds were measured. Sometimes morphological data of a male were not available for a particular season. In such cases I used morphological data from the previous or next season, assuming that birds stop growing when they are adult, and wing length and tarsus length stay constant after reaching adulthood. Weight could vary within males and between seasons, depending on food abundance and the time within a season when measurements were taken. To test if the recorded morphological data stayed constant between seasons, correlations were calculated for morphological data of 14 males that were caught in two or three of the three analysed seasons. For one male that was caught in all three seasons, one randomly chosen season was excluded. Thus correlations could be performed between morphological data taken the first time and the second time. All data were normally distributed (Kolmogorov-Smirnov: all $p > 0.12$). The correlation between morphological measurements of individual males in different seasons was significant for wing length ($r = 1.000$, $B = 0.996$, $p < 0.0005$), tarsus length ($r = 0.999$, $B = 0.986$, $p < 0.0005$), and weight ($r = 0.999$, $B = 0.974$, $p < 0.0005$), indicating that male morphology did not vary much between seasons. Thus it was safe to use data from the previous or next season if they were not available for a particular season and male.

The exact age of a male was known only if it was ringed as a nestling or one year old male during breeding season in non-breeding plumage. The age of males which were in full breeding plumage at their first capture could not be estimated accurately, only a minimum age of two years in the year of their first capture could be determined. For an analysis of differences between age classes two groups were formed. The first group comprised males that were two years old, the second group comprised at least three years old males. Thus the second group included males whose exact age was not known, but who were at least three years old. For 2000/2001, sample size was 25, but only two males were known to be two years old and statistics could not be performed. For 2001/2002, sample size was 25 with 5 males of two years of age and for 2002/2003 sample size was 26 with 6 males of two years of age; thus statistics could be calculated for both of the two seasons.

3.3 Mating success, nest-building effort and skills, and nest characteristics

Male mating success was measured as the number of accepted nests, the number of eggs, the number of hatchlings, and the number of fledglings. Since all four measurements of male mating success correlated significantly with each other (Spearman-Rho correlations: all $p < 0.0005$, all $r_s > 0.67$), I used only the number of accepted nests per male as measurement for mating success in the following analyses.

Several parameters were obtained to describe male nest-building activity. The number of nests was defined as the number of completed nests per male for the whole season. The proportion of abandoned nest-building attempts was defined as the number of unfinished nests (abandoned nest-building attempts) divided by the total number of nests per male for the whole season including unfinished and finished nests. An efficiently building male should have only few abandoned nest-building attempts. The proportion of unusable nests was defined as the number of unusable nests divided by the total number of completed nests. In the following, nest-building skills were defined as the proportion of abandoned nest-building attempts and the proportion of unusable nests. Territory tenure was measured in days from the beginning of the first nest until the date of the last nest finished or accepted. This was a very conservative measure and was probably lower than the actual time of territory tenure, but the mere sighting of a male in the colony was regarded as not reliable enough to assign territoriality status to it. Nest-building effort was measured as days needed to build one nest (building time) and days since finishing the last nest when a new nest was started (building delay). Parameters describing nest-building effort were recorded for each nest separately; then, median values were calculated for each male.

In Red Bishops, a typical nest consists of an entrance located on the side of the upper half of the nest, a short entrance roof overlap and the lower half of the nest serving as the main breeding chamber (see schematic drawing in Figure 1). In 2002/2003, a total of 210 nests were measured from October 2002 to February 2003 to investigate whether certain nest characteristics affect nest acceptance by females or nest durability. Measurements taken were breeding chamber depth, width and height (the breeding chamber was defined as the lower half of the nest), entrance ring width and height, and entrance roof overlap (see Figure 1). In addition, I measured breeding chamber density by holding a piece of thin wire of 1 cm length on the backside of the breeding chamber and counting the number of fibres going across this wire.

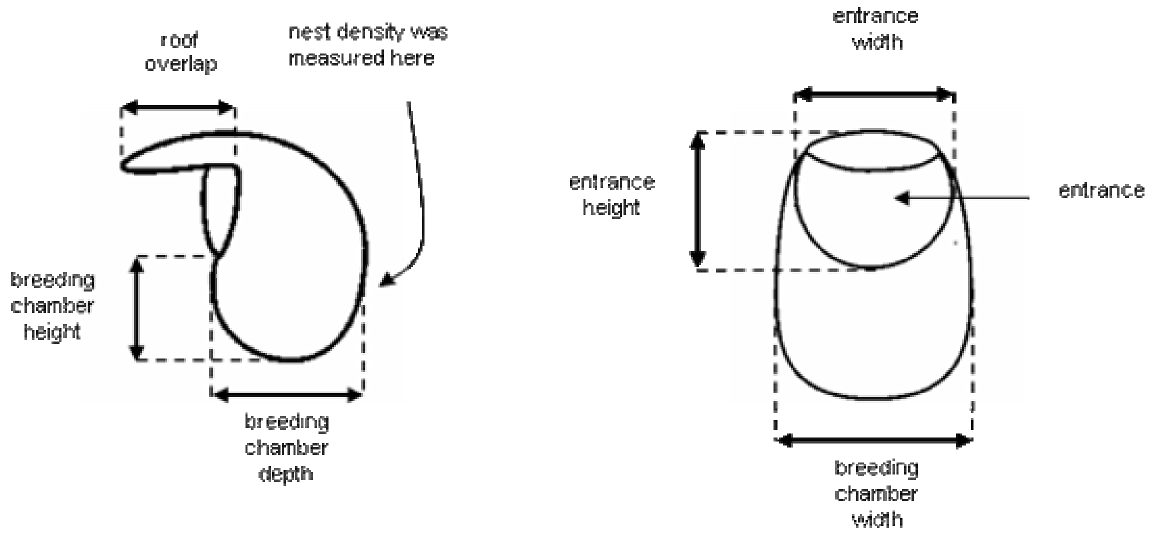


Figure 1 Illustration of the nest measurements taken in 2002/2003.

Nests were measured 4 days after they were started. Most nests were finished within 4 days (see Chapter 2), so they should have been in a comparable state. If a nest was not yet finished after 4 days (i.e. the breeding chamber was not yet closed) it was not measured, but again inspected after two more days. If the nest was finished by then it was measured, otherwise it was no longer considered for nest measurements. Based on the measurements obtained I calculated the following variables. The volume of the breeding chamber was approximated as the volume of a half ellipsoid and calculated with $V = \frac{2}{3} \pi * r_1 * r_2 * r_3$, were r_1, r_2, r_3 are the radii of the ellipsoid. The “narrowness” of a breeding chamber (i.e. the ratio of horizontal cross-sectional area to height) was calculated by narrowness = width * depth / height, with large values indicating a broad and low (i.e. “stocky”) nest and small values indicating a narrow and high (i.e. “slender”) nest. To describe breeding chamber deformation, I used breeding chamber width and depth and divided the larger value by the smaller value. An even nest would have a value of one and any deformation would be represented by a value larger than 1. Likewise, deformation of the entrance ring was calculated based on the proportion of entrance ring width and height, again dividing the larger value by the smaller value. To describe entrance ring size I calculated width * height of the entrance ring. This value would of course describe the area of a rectangle and not of a circle, but because sizes were all calculated in the same way and then compared, it does not matter if all values would be multiplied by a constant ($^1/_4\ \pi$) or not.

While the nest frame is built by males only, females modify nests after they accepted a nest frame (i.e. laid an egg in it) by lining the inside of the nest frames and padding the breeding chamber with reed fibres and soft plant seeds. Nest modification by females occurs after eggs were laid into the nest (Skead 1956, Friedl 2004).

Only nests measured in the first month were included in the analysis investigating effects of nest characteristics on nest acceptance by females, because female breeding activity concentrated on the first month in this breeding season (see Chapter 2). For nests that were built later the probability of being accepted by a female was very low even if the nest characteristics matched possible female preferences. Thus, the inclusion of nests built after the breeding peak in the first month of the breeding season would have hampered the detection of female preferences for certain nest characteristics.

For the analysis of nest characteristics affecting nest durability I defined durability as a nest becoming unusable or not. Nests were considered unusable if they became unusable within 14 days after being finished (see definition for the proportion of unusable nests per male). Nests that were destroyed by other weaverbirds were excluded from this analysis. The aim was to include only nests that became unusable because of faults in nest architecture, and compare these to nests that lasted longer.

2.4 Statistical analyses

For the analysis of reproductive effort (number of nests built) and mating success (number of nests accepted), general linear models (GLMs) were calculated. All models were saturated based on type III sum of squares. Nest characteristics were analysed with binary logistic regressions. Parameter estimates with B coefficients were calculated to determine the direction of the influence of independent covariables. All tests were performed using statistical software package SPSS 11.

4 Results

4.1 Variation of breeding activity among seasons

There were considerable differences between seasons in various parameters describing nest-building behaviour and mating success (Table 1). The high proportion of destroyed or deformed nests in the last season is probably due to the presence of Blackheaded Herons (*Ardea melanocephala*), which were absent or scarce in previous seasons. Many nests were found ripped open and emptied if they contained eggs or nestlings, a typical sign for predation by herons. Some parameters also showed high variation within seasons, notably the proportion of abandoned nest-building attempts and the average building time for a nest.

Table 1 Descriptive statistics for parameters describing male nest-building behaviour, reproductive effort and mating success. The last column shows p-values of Kruskal-Wallis tests for differences between seasons.

	breeding season	median	interquartile	range	standardised variance (variance/mean^2)	Kruskal-Wallis p
number of nests built	2000/2001	12	5.75 - 14.25	1 - 24	0.258	0.011
	2001/2002	7.5	5 - 11	1 - 18	0.231	
	2002/2003	7	4 - 12	1 - 21	0.314	
proportion of abandoned nests building attempts	2000/2001	0.063	0 - 0.127	0 - 0.29	1.161	< 0.0005
	2001/2002	0.194	0.125 - 0.333	0 - 0.67	0.486	
	2002/2003	0.077	0 - 0.167	0 - 0.5	1.463	
proportion of deformed or destroyed nests	2000/2001	0.200	0.059 - 0.318	0 - 0.5	0.631	< 0.0005
	2001/2002	0.118	0 - 0.250	0 - 0.57	0.933	
	2002/2003	0.412	0.333 - 0.563	0 - 1	0.222	
number of accepted nests	2000/2001	7	3 - 10	0 - 20	0.407	< 0.0005
	2001/2002	4	2 - 6	0 - 11	0.400	
	2002/2003	2	1 - 5	0 - 10	0.607	
proportion of accepted nests	2000/2001	0.594	0.500 - 0.723	0 - 1	0.102	< 0.0005
	2001/2002	0.571	0.400 - 0.709	0 - 1	0.145	
	2002/2003	0.333	0.250 - 0.500	0 - 1	0.357	
average building time for a nest	2000/2001	0.5	0 - 2	0 - 4	1.306	< 0.0005
	2001/2002	2	2 - 2	0 - 8	0.447	
	2002/2003	1	0 - 2	0 - 4	1.240	
average delay between building two consecutive nests	2000/2001	4	4 - 7	0 - 19	0.387	0.23
	2001/2002	4	2 - 6	0 - 19	0.534	
	2002/2003	4	2 - 6	0 - 22	0.703	

4.2 Male mating success and reproductive effort

In the following, the influence of the above mentioned aspects of nest-building behaviour – the proportion of unusable nests, the proportion of abandoned nest-building attempts, building time, and building delay – on mating success in terms of the number of nests accepted and reproductive effort in terms of the number of nests built was investigated.

4.2.1 Determinants of mating success

Data were analysed for all seasons combined in a general linear model with season as factor and the number of nests built, territory tenure, the proportion of unusable nests, the proportion of abandoned nest-building attempts, building time, and building delay as covariates. The dependent variable investigated was mating success measured in terms of the number of accepted nests. The resulting model showed significant effects of the number of nests built, the proportion of unusable nests, the proportion of abandoned nests, territory tenure and season (see Table 2). This model explained about 82% of the observed variance in mating success measured by female nest acceptance.

Table 2 The results of a general linear model with the number of accepted nests as dependent variable.

Source	B coefficient	sum of squares type III	df	F	partial Eta-square	p
corrected model		1691.493	8	97.208	0.830	< 0.001
intercept	1.735	37.195	1	17.100	0.097	< 0.001
n nests built	0.663	376.260	1	172.985	0.521	< 0.001
p unusable nests	-5.909	152.806	1	70.252	0.306	< 0.001
p abandoned nests	-2.229	12.228	1	5.622	0.034	0.019
territory tenure	-0.020	21.896	1	10.067	0.060	0.002
building time	0.093	1.869	1	0.859	0.005	0.355
building delay	0.028	1.090	1	0.501	0.003	0.480
season	0.876 / 0.218*	14.867	2	3.418	0.041	0.035
error		345.841	159			

adjusted $r^2 = 0.822$, N = 168
*Seasons 2000/2001 and 2001/2002 respectively. B for 2002/2003 is redundant (insufficient degrees of freedom).

Parameter estimates of the resulting model indicated a positive relation of the number of nests built and a negative relation of the proportion of unusable nests, the proportion of abandoned nests, and territory tenure with the number of accepted nests. The negative effect of territory tenure on the number of accepted nests indicates that for males with the same number of nests built, males that built their nests within a shorter time period (i.e. a shorter territory tenure) were more successful than males that built their nests over a longer time period (i.e. longer territory tenure).

4.2.2 Determinants of reproductive effort

As above (Table 2) and previously shown (Friedl & Klump 1999, Lawes *et al.* 2002), the number of nests built is the most important determinant of male mating success. The following analysis should reveal determinants of reproductive effort (number of nests built). A general linear model was calculated with reproductive effort as dependent variable, season as factor and territory tenure, the proportion of unusable nests, the proportion of abandoned nest-building attempts, building time, and building delay as covariates (see Table 3). The resulting model explained about 75% and revealed significant effects of territory tenure, building time, building delay and season. The effect of the proportion of abandoned nest-building attempts was nearly significant. A high number of nests built resulted from long territory tenure, short building time and short building delay. As can be seen in Table 1, the number of nests built per male was highest in the first of the analysed seasons, also indicated by the positive B coefficient in Table 3.

Table 3 The results of a general linear model with the number of built nests as dependent variable.

Source	B coefficient	sum of squares type III	df	F	partial Eta-square	p
corrected model		2775.137	7	74.156	0.764	< 0.001
intercept	4.08	289.328	1	54.119	0.253	< 0.001
p unusable nests	0.318	0.433	1	0.083	0.001	0.774
p abandoned nests	-2.855	20.546	1	3.843	0.023	0.052
territory tenure	0.101	1621.181	1	303.244	0.655	< 0.001
building time	-0.426	40.935	1	7.657	0.046	0.006
building delay	-0.451	420.963	1	78.742	0.330	< 0.001
season	1.917 / 1.026*	67.231	2	6.288	0.073	0.002
error		855.381	160			

adjusted $r^2 = 0.754$, N = 168
*Seasons 2000/2001 and 2001/2002 respectively. B for 2002/2003 is redundant (insufficient degrees of freedom).

4.3 Morphological measures

General linear models with a reduced dataset using only males for which wing length, tarsus length and weight were available (N = 57), showed no significant effects of any of these morphological measures on the number of accepted nests (Table 4) or the number of nests built (Table 5). Significant determinants for mating success and reproductive effort were similar to the model with the full dataset, with the exception that the determinants with the weakest significance in the full models were dropped in the models with the reduced dataset.

Table 4 The results of a general linear model with the number of accepted nests as dependent variable, including male morphology.

Source	B coefficient	sum of squares type III	df	F	partial Eta-square	p
corrected model		599.876	11	21.320	0.839	< 0.001
intercept	4.913	0.524	1	0.205	0.005	0.653
n nests built	0.715	143.201	1	55.983	0.554	< 0.001
p unusable nests	-6.277	49.485	1	19.346	0.301	< 0.001
p abandoned nests	-1.512	1.725	1	0.674	0.015	0.416
territory tenure	-0.037	18.435	1	7.207	0.138	0.010
building time	-0.196	1.985	1	0.776	0.017	0.383
building delay	-0.020	0.096	1	0.038	0.001	0.847
wing length	-0.027	0.111	1	0.044	0.001	0.836
tarsus length	0.076	0.250	1	0.098	0.002	0.756
weight	0.055	0.288	1	0.113	0.002	0.739
season	0.202 / 0.058*	67.231	2	0.037	0.002	0.964
error		115.106	45			

adjusted $r^2 = 0.800$, N = 57

*Seasons 2000/2001 and 2001/2002 respectively. B for 2002/2003 is redundant (insufficient degrees of freedom).

Table 5 The results of a general linear model with the number of built nests as dependent variable, including male morphology.

source	B coefficient	sum of squares type III	df	F	partial Eta-square	p
corrected model		1061.909	10	17.444	0.791	< 0.001
intercept	-2.397	0.033	1	0.005	< 0.001	0.941
p unusable nests	2.135	5.847	1	0.961	0.020	0.332
p abandoned nests	-3.840	11.590	1	1.904	0.040	0.174
territory tenure	0.114	434.011	1	71.297	0.608	< 0.001
building time	-0.217	2.445	1	0.402	0.009	0.529
building delay	-0.649	150.384	1	24.704	0.349	< 0.001
wing length	0.231	8.242	1	1.354	0.029	0.251
tarsus length	-0.494	11.108	1	1.825	0.038	0.183
weight	-0.021	0.042	1	0.007	< 0.001	0.934
season	2.258 / 1.149*	22.120	2	1.817	0.073	0.174
error		280.021	46			

adjusted r^2 = 0.746, N = 57
*Seasons 2000/2001 and 2001/2002 respectively. B for 2002/2003 is redundant (insufficient degrees of freedom).

4.4 Nest characteristics

193 nests of 449 nests completed in the season 2002/2003were measured. Of all analysed nest characteristics, breeding chamber density and entrance overlap showed the largest standardised variance and breeding chamber deformation the least variance (Table 6). Males differed in respect to entrance roof overlap and breeding chamber narrowness (Table 6).

Table 6 Descriptives for the different analysed nest characteristics, and p-values of Kruskal-Wallis-tests for differences between males (N = 52).

	median	interquartile	Range	standardised variance (variance/mean^2)	Kruskal-Wallis p
entrance deformation	1.20	1.10 - 1.33	1.00 - 3.25	0.0473	0.788
entrance roof overlap (cm)	4.00	3.5 – 4.5	0.5 – 6.0	0.0673	0.016
entrance size (cm^2)	24.75	20.25 – 27.50	10.00 – 39.00	0.0425	0.204
breeding chamber narrowness (cm)	10.23	9.15 – 11.46	5.50 – 16.36	0.0328	0.037
breeding chamber deformation	1.07	1.06 - 1.14	1.00 - 1.60	0.0095	0.581
breeding chamber density (fibres * cm^{-1})	10.58	8.67 - 12.00	2.00 - 26.00	0.0844	0.192
breeding chamber volume (cm^3)	181.43	161.27 - 203.78	87.11 - 283.73	0.0354	0.136

4.4.1 Nest characteristics and nest acceptance by females

The objective was to analyse nest characteristics that can be ascribed to males only, and were not altered or caused by females. Females also engage in nest-building, although probably only after they accepted a nest frame, i.e. laid an egg in it. The inside of these frames gets lined out and the breeding chamber gets enhanced and thickened by females. It is unknown, when exactly females start modifying the nest frame, some time after laying the first egg, after laying the whole clutch or even before laying an egg. Therefore I had to confirm that the analysed nest characteristics could be ascribed to males only, even if a nest was already accepted when measured or became accepted a few days after being measured. To investigate the possibility that females alter a nest before they lay eggs in it, correlations between the measured nest characteristics and the number of days elapsed between measuring and acceptance in terms of eggs laid were performed. The assumption was that nest modification by females prior to egg-laying would be detectable if nests were measured immediately before they were accepted, but not apparent for nests that were accepted only much later after being measured. Nests that contained already one or more eggs when measured were not included in this test. None of the measured nest characteristics correlated significantly with the time elapsed between measuring and acceptance of a nest (Nonparametric correlations:

all $p > 0.18$). Thus, no modifications on nests by females prior to egg-laying were apparent and measured nest characteristics were fully attributable to males only.

Some nests were already accepted when measured, and to test if these nests could be included in an analysis of effects of nest characteristics on nest acceptance by females, I tested if characteristics of nests already accepted when measured were altered by females. Two groups of nests were compared, one group comprising already accepted nests ($N = 20$), another group comprising nests that were accepted after measuring ($N = 45$). Nests that were already accepted when measured had a narrower and higher breeding chamber (Mann-Whitney-test: $Z = -3.02$, $p = 0.003$). There were no significant differences between other nest characteristics of already accepted and later accepted nests (all $p > 0.25$).

To analyse effects of nest characteristics on nest acceptance by females, nests were assigned to two categories, with the first group comprising nests that were accepted ($N = 57$), and the second group comprising nests that were not accepted ($N = 136$).

A binary logistic regression with acceptance as dependent variable showed significant effects of breeding chamber density ($B = 0.281$, Wald = 18.13, $p < 0.0005$). A higher breeding chamber density increased the likelihood of a nest becoming accepted. Additionally, a larger entrance roof overlap increased the likelihood of nest acceptance ($B = 0.056$, Wald = 8.29, $p = 0.004$).

4.4.2 Nest characteristics and nest durability

As shown above, a higher density of the breeding chamber and a larger entrance roof overlap increased the likelihood of a nest to become accepted. This result could be explained by female preferences for high nest durability or the fact that some nests did not last long enough to become accepted. A higher density of the breeding chamber and a larger entrance roof overlap could increase overall nest stability and consequently nest durability.

In a first step, only nests that were never accepted were analysed. To test for effects of nest characteristics on nest durability I performed a stepwise binary logistic regression with low or high durability as dependent variable and nest measurements as independent variables. Low durability was assigned to nests that became unusable within two weeks after being finished and high durability was assigned to nests that lasted longer than two weeks, equal to the definition of unusable nests mentioned in Methods. The only parameter included in the final model was entrance roof overlap (Wald = 3.59, p = 0.058) with a smaller entrance roof overlap increasing the likelihood of low durability (B = -0.113). Although the Wald statistic was not significant, the omnibus-test for the coefficient of the model was significant (CHI-square = 4.39, df = 1, p = 0.036), when entrance roof overlap was included. See Table 7 for nest characteristics not included in the model.

Table 7 Stepwise binary logistic regression if never accepted nests became unusable within two weeks or not. Displayed are scores and p-values of the variables not included in the model after entrance roof overlap was included.

	score	p
entrance deformation	0.381	0.537
entrance size (cm^2)	1.133	0.287
breeding chamber narrowness (cm)	0.037	0.848
breeding chamber deformation	0.640	0.424
breeding chamber density (fibres * cm^{-1})	1.231	0.267
breeding chamber volume (cm^3)	3.376	0.066

In another analysis, only accepted nests were regarded. One group comprised nests that became unusable while they contained eggs or nestlings (N = 13), whereas the other group of nests lasted at least as long as they contained eggs or nestlings (N = 41). Nests that were destroyed by cape or masked weavers while containing eggs or nestlings were excluded from this analysis, therefore the number of nests in this analysis is smaller than the total number of accepted nests. A stepwise binary logistic regression with group assignment as dependent variable and nest characteristics as independent variables included only breeding chamber density (Wald = 4.57, p = 0.033). Less densely woven nests were more likely to become unusable while containing eggs or nestlings resulting in breeding failure (B = -0.318).

See Table 8 for nest characteristics not included in the model. The omnibus-test for the coefficient of the model was significant (Chi-square = 5.883, df = 1, p = 0.015), when breeding chamber density was included in the model.

Table 8 Stepwise binary logistic regression if accepted nests became unusable or not while containing eggs or nestlings. Displayed are scores and p-values of the variables not included in the model after breeding chamber density was included.

	score	p
entrance deformation	2.256	0.133
entrance overlap (cm)	0.069	0.793
entrance size (cm^2)	2.123	0.145
breeding chamber narrowness (cm)	1.517	0.218
breeding chamber deformation	1.306	0.253
breeding chamber volume (cm^3)	0.204	0.651

4.5 Male age, mating success and reproductive effort

To investigate effects of age on mating success, reproductive effort and nest-building behaviour, two year old males were compared with at least three year old males. This analysis could only be conducted for the seasons 2001/2002 and 2002/2003 (see Methods). Results are summarised in Table 9.

Young males had fewer accepted nests in 2001/2002 and in 2002/2003. To test if this effect was still apparent when the influence of the number of nests built was excluded, the residuals of the regression of the number of accepted nests against the number of nests built for two year old males and older males were compared. No difference was found between young and old males with regard to the residuals of the regression of the number of accepted nests against the number of nests built.

Male mating success was influenced by the proportion of unusable nests and the proportion of abandoned nest-building attempts. Both parameters describe aspects of nest-building skills. For both 2001/2002 and 2002/2003, age classes differed neither in the proportion of abandoned nest-building attempts nor in the proportion of unusable nests (Mann-Whitney-U tests: all $p > 0.07$). In 2001/2002, young males built fewer nests (Mann-Whitney-U = 16, $p = 0.02$), as was found in 2002/2003 (Mann-Whitney-U = 6.5, $p = 0.001$).

As shown in 4.2.2, reproductive effort in terms of the number of nests built depended on territory tenure, building time and building delay. Two year old males had shorter territory tenure times (Mann-Whitney-U = 19.5, $p = 0.014$) than older males in 2002/2003. There was no difference in 2001/2002. Two year old males did not differ in building time from older males (both p > 0.2). In 2001/2002, two year old males had longer building delays than older males (Mann-Whitney-U = 12, p = 0.008), but not in 2002/2003.

Table 9 Comparison between 2 years old males and older males with regard to nest-building behaviour and territory tenure for 2001/2002 and 2002/2003.

					Mann-Whitney	
	season	age	median	range	U	p
n nests built	2001/2002	2 yrs old > 2 yrs	4 10	3 – 11 4 – 18	16.0	0.020
	2002/2003	2 yrs old > 2 yrs	4 10	1 – 6 4 – 21	6.5	0.001
n nests accepted	2001/2002	2 yrs old > 2 yrs	2 6	1 – 4 2 – 10	8.5	0.004
	2002/2003	2 yrs old > 2 yrs	1 3.5	0 – 3 1 – 10	18.5	0.010
residuals n nests accepted	2001/2002	2 yrs old > 2 yrs	-0.432 0.344	-2.515 – 0.662 -2.151 – 3.120	32.5	0.234
	2002/2003	2 yrs old > 2 yrs	-0.291 0.653	-1.421 – 1.209 -3.497 – 2.985	41	0.247
p unusable nests	2001/2002	2 yrs old > 2 yrs	0.167 0.087	0 – 0.385 0 – 0.385	43.5	0.653
	2002/2003	2 yrs old > 2 yrs	0.400 0.406	0 – 1 0.200 – 0.700	52.0	0.625
p abandoned nests	2001/2002	2 yrs old > 2 yrs	0.250 0.160	0.154 – 0.667 0 – 0.429	24.0	0.077
	2002/2003	2 yrs old > 2 yrs	0.100 0.130	0 – 1 0 – 1	58.0	0.900
territory tenure	2001/2002	2 yrs old > 2 yrs	68 65	46 – 89 36 – 97	45.0	0.733
	2002/2003	2 yrs old > 2 yrs	19.5 92.5	9 – 107 12 – 120	19.5	0.014
building time	2001/2002	2 yrs old > 2 yrs	2 2	0 – 2 0 – 4	47.5	0.820
	2002/2003	2 yrs old > 2 yrs	1.5 0	0 – 4 0 – 4	39.0	0.171
building delay	2001/2002	2 yrs old > 2 yrs	7 2.5	4 – 14 0 – 10	12.0	0.008
	2002/2003	2 yrs old > 2 yrs	4.5 4	0 – 8 0 – 6	51.5	0.599

2001/2002: N = 5 males 2 years old, N = 20 males older than 2 years
2002/2003: N = 6 males 2 years old, N = 20 males older than 2 years

To test if young males built nests with different characteristics than nests of older males, mean values of nest characteristics were calculated for each male.

Because not all nests were measured, nest characteristics for one two year old male and one older male were not available, reducing the sample to five two year old males and 19 older males. Based on the analysed nest characteristics, nests of young males were not distinguishable from nests of older males (Table 10) (all p > 0.1).

Table 10 Mann-Whitney-U tests for differences between nest characteristics of 5 two year old males and 19 older males.

				Mann-Whitney	
		median	range	U	p
entrance deformation	2 yrs old > 2 yrs	1.196 1.191	1.056 – 1.375 1.081 – 1.750	42	0.696
entrance overlap (cm)	2 yrs old > 2 yrs	4.0 3.917	3.5 – 5.0 2.6 – 4.6	38	0.498
entrance size (cm^2)	2 yrs old > 2 yrs	21.38 24.50	17.00 – 33.00 13.00 – 29.25	30	0.214
breeding chamber narrowness (cm)	2 yrs old > 2 yrs	10.67 9.78	9.50 – 12.90 7.40 – 12.10	26	0.126
breeding chamber deformation	2 yrs old > 2 yrs	1.067 1.095	1.000 – 1.231 1.052 – 1.259	31	0.241
breeding chamber density (fibres * cm^{-1})	2 yrs old > 2 yrs	9.667 10.889	8 – 12 8 – 15	31	0.240
breeding chamber volume (cm^3)	2 yrs old > 2 yrs	187.5 179.3	149.7 – 201.1 147.3 – 230.7	44	0.804

5 Discussion

5.1 Nest-building effort and skills

The importance of the number of nests built for reproductive success in male *Euplectes orix* was further confirmed by this study, as established by Friedl & Klump (1999) and Lawes *et al.* (2002). In a closely related species *Euplectes macrourus* the number of nests built was also the best predictor for male mating success (Savalli 1994a). For the same species Savalli (1994b) found that males with experimentally shortened tails were at a disadvantage in male-male competitions over territories. Unfortunately, it is not known if territory tenure also influenced the number of nests built in *Euplectes macrourus* as in *Euplectes orix*. In another closely related polygynous species, the Red-shouldered Widowbird (*Euplectes axillaris*), the number of accepted (active) nests was related to the number of nests built (cock's nests) by a male, and the number of nests built was not related to territory size or quality (Pryke & Andersson 2003). Another species with a high level of polygyny is the Wren (*Troglodytes troglodytes*), common in northern temperate regions. The observed level of polygyny is much lower (maximum 9 breeding attempts by a male) than in Red Bishops (maximum 20 breeding attempts by a male), but nevertheless there are some similarities. Direct female choice seems to be absent, instead the number of nests built determines male mating success (Evans & Burn 1996). As in Red Bishops, the nest frame is built by males only, and the inner lining and padding is done by females. It is not known what aspects of nest-building skills determine nest-building rate in this species, but territory quality in terms of nest survival probability was related to male tail length (Evans & Burn 1996). Thus, male-male competition over high quality territories might affect male mating success in the Wren. Long-billed Marsh Wrens are another polygynous songbird species where only the number of nests influenced male mating success (Verner & Engelsen 1970). In this species, male mating success varies between 0 and 2 females attracted as mates per season. Territory characteristics like size and several vegetational parameters were not related to male mating success (Verner & Engelsen 1970). However, quality and skills of male nest-building were not investigated and therefore the influence of male nest-building quality and skills remain unknown for these species.

This study demonstrates how nest-building skills and nest-building effort related to male reproductive effort and mating success in the Red Bishop. Not only the mere number of completed nests, but also some qualitative aspects of nest-building behaviour influenced mating success. A smaller proportion of unusable nests and a smaller proportion of abandoned nest-building attempts enhanced reproductive success independent of the number of nests built by a male and thus reflected some aspect of the quality of nests built by a male. A nest was designated unusable if it was not accepted and destroyed or deformed within two weeks after completion. Thus a smaller proportion of unusable nests increased the number of available nests at any given time, independent of the number of nests built. This could explain the observed large influence of the proportion of unusable nests on mating success independent of the number of nests built.

A Red Bishop nest could become unusable because a cape weaver destroyed that nest, in which case the builder has no influence on the nest quality. If a Cape Weaver chooses to tear down a Red Bishop nest and build his own nest in that place, there is nothing a Red Bishop can do against it due to the size difference between the two species (Friedl 2004), but choose a nest site in safe distance from a Cape Weaver's territory. Further on, a nest could also become unusable because it was squashed. This could happen because the nest was fastened on reed or bulrush stems that were too weak to support the nest or the nest itself was to weak to withstand the pressure exerted on it by the stems moving in the wind. These events were under influence of the builder by choosing the right stems and building a stable nest. Thus the proportion of unusable nests partially described male nest-building skills. The proportions of nests destroyed by Cape Weavers of all unusable nests were 13%, 19%, and 21% for seasons 2000/2001, 2001/2002, and 2002/2003 respectively. The Cape Weavers at the study colony were all building nests over open water, whereas red bishops built nests over open water as well as on dry ground on the edge of the pond. Consequently territory location could play some role in determining male mating success. After Friedl (in press) nests built above water were less likely to be accepted, but chances for a partial or complete clutch or brood loss did not differ between nests built on dry ground and nests built on water. Another aspect of nest-building skills was the proportion of abandoned nest-building attempts. The smaller the proportion of abandoned nest-building attempts the higher was male reproductive success. This result could be explained by a male's ability to fasten the first fibres on a reed or bulrush stem. A male encountering difficulties in attaching the first fibres might abandon this nest-building attempt and switch to another site. Based on the total number of nests, unfinished and finished, a smaller proportion of abandoned nest-building attempts would increase the number of completed nests, but the positive effect of a smaller proportion of abandoned nest-building attempts was apparent even if the influence of the number of completed nests was eliminated. Interestingly, the proportion of abandoned nest-building attempts did not influence the number of nests built, but it must be cautioned that this result was nearly significant ($p = 0.052$). Because the influence of the proportion of abandoned nest-building attempts was much stronger on the number of accepted nests, the proportion of abandoned nest-building attempts might reflect a general pattern of building a nest that is also apparent in completed nests.

Reproductive effort as the number of nests a male built during a season was predicted by territory tenure and nest-building effort in terms of building delay and building time. The duration of building delay as well as the time needed to build a nest might depend on the physical fitness of a male. Dolnik (1991) studied energy expenditure needed for nest-building in detail and found that energy spent for nest-building amounts to 1.5 to 4.9 times of the basal metabolism. The energy needed to build a nest suffices for the production of 0.5 to 2.7 clutches. The slope of energy expenditure per nest is similar to the slope of energy cost of locomotion. This matches the observation of Collias & Collias (1984) who stated that energy costs for male weaverbirds of building a nest can be high depending on the proximity of nest-building material to the nest site, and weaker males might need more time to recover from the effort spent on nest-building. Collias & Collias (1984) suggested that even with close proximity to nest-building material and consequently reduced energy expenditure, a male weaverbird needs a time delay between building two consecutive nests to restore its energy balance. The number of nests built by a male also depended on territory tenure. Territory tenure in turn might depend on a male's fighting ability with regard to its neighbours, since most of male-male interactions involve neighbours (Craig 1974).

I found no significant influence of the measured male morphological characteristics on mating success as measured in terms of the number of nests accepted or mating effort as measured in terms of the number of nests built. These results are in accordance with available data on other closely related species. Both in the Red-collared Widowbird (*Euplectes ardens*) and in the Yellow-shouldered Widowbird (*Euplectes macrourus*) mating success was unrelated to male body size or weight (Savalli 1994a, Pryke *et al.* 2001). In addition, Evans & Burn (1996) found that body mass was also unrelated to mating success in the Wren, a polygynous species with a mating system similar to the one of the Red Bishop (see above).

5.2 Nest characteristics

Of the analysed nest characteristics only nest density in terms of the number of fibres per cm in the wall of the breeding chamber and entrance roof overlap proved to be important for the acceptance of a nest by a female. Both a denser nest and a larger entrance roof overlap might increase nest stability and protection of nestlings from rain. These data show that females prefer stronger and more stable nests, because nests that became unusable while they still contained eggs or nestlings were less densely woven than nests that lasted at least as long as they contained eggs or nestlings. This result matches the fact that male mating success was influenced by the proportion of nests that became unusable within two weeks independent of the number of nests built. In regard to nest density Red Bishops seem to differ from Village Weavers since closeness of weave did not influence mating success in the latter species (Collias & Victoria 1978). However, closeness of weave as measured by Collias & Victoria (1978) is not equivalent to nest density as defined here, because closeness of weave was estimated on the number of loose loops projecting from the surface of the nest. A similar result was found for Penduline Tits (*Remiz pendulinus*), a polygynous songbird of northern temperate areas. Larger and thicker nests were better insulated and preferred by females (Hoi *et al.* 1994). However, insulation is not likely to play a role in nests subtropical birds. In Red Bishops, accepted nests are probably exposed to considerable strain by the weight of the incubating female and 2-4 nestlings, and a more densely woven nest might better withstand this strain. The significant result that nests that became unusable while they still contained eggs or nestlings were less densely woven than nests that lasted at least as long as they contained eggs or nestlings is actually surprising. Either females did not choose accurately with regard to nest density or sometimes lower quality nests are chosen because of limited supply. Since nest density in terms of the number of fibres per cm affects the durability of nest containing eggs or nestlings, an inaccurate choice by females would be surprising because of its consequences for their breeding success. In case of limited supply females could compete for good nests, with more aggressive females obtaining better nests. But on any given day on which nests were accepted, the number of available nests (not yet accepted, still usable and not older than two weeks) was at least double the number of accepted nests. Therefore any female competition for good and durable nests, i.e. nests with a high density of fibres, could have been of only minor importance. Anyway, female competition for nests has not been recorded in literature describing the biology and breeding behaviour of Red Bishops (Skead 1956, Emlen 1957, Woodall 1971, Craig 1974, Friedl 2004). Therefore I favour the explanation of poor accuracy of female choice for nest density in terms of the number of fibres per cm. This assumed poor accuracy can be explained by general theories on signal detection (e.g. Johnstone & Grafen 1992, De Jaegher 2003).

The importance of the entrance roof overlap for nest durability could not raise questions about the accuracy of female choice for nests, because it had an effect on durability only in never accepted nests. Accordingly, females chose accurately with regard to entrance roof overlap and there were always enough nests with a sufficiently large entrance roof overlap available. This would explain why no effect of entrance roof overlap on the durability of accepted nests was found. A larger entrance roof overlap can enhance nest stability and durability because a larger roof overlap can be attached to more, additional stems.

Nests that were already accepted when they were measured had a narrower and taller breeding chamber than other nests. This could be attributed to females altering the shape of a nest merely by sitting in it. Although females work extensively on nests by applying a heavy lining after laying eggs, no changes in nest density as a result of female work could be detected. Apparently nests were measured early enough, before female work on these nests was detectable.

The contribution of certain nest characteristics to breeding success has been demonstrated earlier for other bird species. In Tree Swallows (*Tachycineta bicolor*), an influence of nest characteristics has been reported by Lombardo (1994), revealing a complex relation between breeding success, nest size and insulation and the number of nestlings in a nest. Similarly, Alabrundzinska *et al.* (2003) suggest several contradictory pressures on nest size and composition for optimal breeding success in Great Tits (*Parus major*). Nest size and bottom thickness were positively related to breeding success in Penduline Tits (*Remiz pendulinus*), and females prefered larger nests (Hoi *et al.* 1994).

5.3 Age and reproductive success

There are three broad groups of hypotheses explaining age-related differences in reproductive success (for a review see Forslund & Pärt (1995). The first possible explanation for differences in male reproductive success relative to age is given by a group of hypotheses that assume a positive link between reproductive success and viability, thus selecting for males with higher reproductive success. Consequently, older cohorts would have higher reproductive success. The second group of hypothesis assumes that males acquire experience with years, enabling them to increase reproductive success over seasons. Such experiences could be familiarity with food sources in a breeding habitat, improved foraging ability or breeding experience, or quality and skills of nest-building. The third general explanation after Forslund & Pärt (1995) assumes optimization of reproductive effort with age. If survival probability decreases with age, optimal reproductive effort will increase, because there is no reason to save resources for a future that does no longer exist. But Forslund & Pärt (1995) caution that this hypothesis would imply rather constant reproductive effort in the first years of live, only increasing when survival probability becomes markedly smaller in old age. Age-related changes in reproductive success are well known for birds (e.g. Evans 1997, Espie *et al.* 2000, Penteriani *et al.* 2003), but not yet documented for weaverbirds. If age had any influence on reproductive effort (number of nests built) and mating success (number of nests accepted), a linear relation where reproductive effort and mating success steadily increase with age was regarded very unlikely (Forslund & Pärt 1995). Old males could perform poorer than younger males because of poorer physical fitness and senescence, whereas young, two years old males in their first breeding season could perform poorer than older males because of lack of experience, but not because of differences in physical fitness. Therefore I compared only young males with older males and did not calculate correlations between age and breeding success or parameters describing nest-building behaviour.

Males older than 2 years showed higher reproductive effort in terms of the number of nests built and higher mating success than two year old males. To test if differences between age classes in Red Bishops with regard to mating success can be partially explained by the selection hypothesis assuming a genetic link between mating success and viability, the first hypothesis mentioned by Forslund & Pärt (1995), several breeding seasons must be compared. Many male Red Bishops return to the same colony in following seasons if they successfully established a territory (Friedl 2004). Thus, changes in the composition of age cohorts of territorial males could be analysed. Young males with low reproductive success would not be expected to be found in following breeding seasons, if the selection hypothesis applies and reproductive success is linked to viability and survival rate. Some evidence against this hypothesis is given by Friedl & Klump (1999), who compared presently territorial males that established a territory in the following season with presently territorial males that were present in the following season, but failed to establish a territory in the following season. Friedl & Klump (1999) found that males that established a territory in the following season built more nests and held their territory for longer in the current season. Thus, males with lower quality defined as the number of nests built, territory tenure and the ability to establish a territory did not die because of reduced viability, ruling out a link between viability and male quality.

The second hypothesis after Forslund & Pärt (1995), assuming an increase in experience and competence, e.g. breeding experience, forage ability, access to resources through early arrival or dominance, could explain the observed differences between young and old males, although underlying causes for the observed difference in the number of nests built were different between seasons. In one season, young males held territories for a shorter time and in another season, young males had longer building delays than older males. These results were not consistent over the two seasons for which statistics could be performed, but might indicate that young males are unable to invest much in reproductive effort whereas no differences were found between age classes with regard to the analysed aspects of nest-building skills in terms of the proportion of unusable nests and the proportion of abandoned nest-building attempts. It seems that male Red Bishops do not show an age-related increase in nest-building skills in terms of the proportion of unusable nests and the proportion of abandoned nest-building attempts, but there might be an increase in reproductive effort.

The difference between two year old males and older males does not match the third group of hypotheses discussed by Forslund & Pärt (1995), the assumption of optimization of reproductive effort, because this effect would rather be apparent in older males and not in males in their first breeding season, as is the case in this study. Because of the observed difference between young males in their first breeding season and older males, it is rather probable that young males in their first breeding season do not invest less in reproductive effort to increase their life expectancy, but are less experienced than older males. This lack of experience does not apply to nest-building skills, but might rather be the case for other skills like foraging ability. Young males would accordingly not hold a territory for a long time, but spend their time foraging.

Clearly, a more detailed analysis is necessary to identify mechanisms leading to differences in reproductive success between age classes in male Red Bishops. Most importantly, changes in reproductive success, nest-building effort and skills over several seasons for individual males must be investigated, as well as between seasons within male variance compared with within season between male variance in reproductive success and nest-building effort and skills. Nests of one year old, non-breeding males look much looser and untidier than nests of breeding males (Friedl 2004), indicating that males have to learn how to build a nest in their first breeding season, similar to Village Weavers (Collias & Collias 1964). However, no differences in nest-building skills analysed in this study were found between two year old males and older males with more experience.

Some light was shed on the importance of nest-building effort and skills for mating success and reproductive effort. Nest-building effort in terms of time needed to build a nest and time delay between finishing a nest and building a new nest determines the number of nests built and might increase with age, as was found for building delay in one season, whereas nest-building skills and ability determine male mating success and do not differ between age classes. The importance of sensory and motor skills in terms of the proportion of abandoned nest-building attempts and the proportion of unusable nests on male mating success is a result not reported so far for weaverbirds. Territory tenure was the strongest determinant of the number of nests built and should be addressed in future studies. Considering the ferocity with which territories are defended, male-male competition for territories might play a role. Fighting ability could influence the outcome of agonistic dyads between males and could be related to the duration for which a territory is successfully defended.

6 References

Alabrundzinska, J., Kalinski, A., Slomczynski, R., Wawrzyniak, J., Zielinski, P., Banbura, J. 2003. Effects of nest characteristics on breeding success of Great Tits *Parus major*. Acta Ornithologica 38: 151-154

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, New Jersey

Barber, I., Nairn, D., Huntingford, F.A. 2001. Nests as ornaments: revealing construction by male sticklebacks. Behavioral Ecology 12(4): 390-396

Borgia, G. 1985. Bowers as markers of male quality. Test of a hypothesis. Animal Behaviour 35: 266-271

Bult, A., Lynch, C.B. 1997. Nesting and fitness: Lifetime Reproductive Success in House Mice Bidirectionally selected for Thermoregulatory Nest-building behaviour. Behavior Genetics 27(3): 231-240

Catchpole, C.K., Slater, P.J.B,. 1995. Bird song. Biological themes and variations. Cambridge University Press, Cambridge

Collias, E.C., Collias, N.E. 1964. The development of nest-building behaviour in a weaverbird. Auk 81: 42-52

Collias, N.E., Collias, E.C. 1984. Nest-building and bird behavior. Princeton University Press, Princeton, New Jersey

Collias, N.E., Victoria, J.K. 1978. Nest and mate selection in the village weaverbird (*Ploceus cucullatus*). Animal Behaviour 26: 470-479

Collis, K., Borgia, G. 1993. The cost of male display and delayed plumage maturation in the Satin Bowerbird (*Ptilonorhynchus violaceus*). Ethology 94: 59-71

Craig, A.J.F.K. 1974. Reproductive behaviour of the male red bishop bird. Ostrich 45: 149-160

De Jaegher, K. 2003. Error-proneness as a handicap principle. Journal of Theoretical Biology 224(2): 139-152

Dolnik, V.R. 1991. Time and energy needed for nest-building. Zoologicheskii Zhurnal 70(4): 97-106

Doucet, S.M., Montgomerie, R. 2003. Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. Behavioral Ecology 14(4): 503-509

Emlen, J.T.Jr. 1957. Display and mate selection in the whydahs and bishop birds. Ostrich 28: 202-214

Espie, R.H.M., Oliphant. L.W., James, P.C., Warkentin, I. G., Lieskea, D.J. 2000. Age-dependent breeding performance in merlins (*Falco columbarius*). Ecology 81(12): 3404-3415

Evans, M.R. 1997. Nest-building signals male condition rather than age in wrens. Animal Behaviour 53: 749-755

Evans, M.R., Burn, J.L. 1996. An experimental analysis of mate choice in the wren: a monomorphic, polygynous passerine. Behavioral Ecology 7(1): 101-108

Ferguson, J.W.H. 1994. Do nest site characteristics affect the breeding success of red bishops *Euplectes orix*? Ostrich 65: 274-280

Forslund, P., Pärt, T. 1995. Age and reproduction in birds – hypotheses and tests. Trends in Ecology and Evolution 10: 374-378

Friedl, T.W.P. 2004. Breeding behaviour of the Red Bishop (*Euplectes orix*): a synthesis and new observations. Die Vogelwarte 42: 177-190

Friedl, T.W.P. in press. Breeding success in a Red Bishop (*Euplectes orix*) colony in the Eastern Cape, South Africa, and its relation to nest site characteristics.

Friedl, T.W.P., Klump, G.M. 1999. Determinants of male mating success in the red bishop (*Euplectes orix*). Behavioural Ecology and Sociobiology 46: 387-399

Hill, G.E., 1999. Mate choice, male quality, and carotenoid-based plumage colouration. In: Proceedings of the 22nd International Ornithological Congress (Adams, N.J., Slotow, R.H., eds). Durban: Birdlife South Africa 1654-1668

Hoi, H., Schleicher, B., Valera, F. 1994. Female mate choice and nest describtion in penduline tits, *Remiz pendulinus*: the importance of nest quality. Animal Behaviour 48: 743-746

Hoi, H., Schleicher, B., Valera, F. 1996. Nest size variation and its importance for mate choice in penduline tits, *Remiz pendulinus*. Animal Behaviour 51: 446-466

Johnstone, R.A., Grafen, A. 1992. Error-prone signalling. Proceedings of the Royal Society London B Biological Science 248(1323): 229-33

Kruuk, L.E.B., Clutton-Brock, T.H., Rose, K.E., Guiness, F.E. 1999. Early determinants of lifetime reproductive success differ between the sexes in red deer. Proceedings of the Royal Society London B Biological Science 266(1429): 1655-1661

Lawes, M., Slotow, R., Andersson, S. 2002. Male nest-building but not display behaviour directly influences mating success in the polygynous Red Bishop, *Euplectes orix*. Ostrich 73(3-4): 87-91

Lindenfors, P., Tullberg, B.S., Biuw, M. 2002. Phylogenetic analyses of sexual selection and sexual size dimorphism in pinnipeds. Behavioural Ecology and Sociobiology 52(3): 188-193

Lombardo, M.P. 1994. Nest architecture and reproductive-performance in tree swallows (*Tachycineta bicolor*). Auk 111 (4): 814-824

Madden, J.R. 2003. Bower decorations are good predictors of mating success in the spotted bowerbird. Behavioural Ecology and Sociobiology 53(5): 269-277

Ostlund-Nilsson, S. 2000. Are nest characters of importance when choosing a male in the fifteen-spined stckleback (*Spinachia spinachia*)? Behavioural Ecology and Sociobiology 48(3): 229-235

Penteriani, V., Balbontin, J., Ferrer, M. 2003. Simultaneous effects of age and territory quality on fecundity in Bonelli’s Eagle *Hieraaetus fasciatus*. Ibis 145(2): E77-E82

Pryke, S.R., Andersson, S. 2003. Carotenoid-based status signalling in red-shouldered widowbirds (*Euplectes axillaris*): epaulet size and redness affect captive and territorial competition. Behavioural Ecology and Sociobiology 53(6): 393-401

Pryke, S.R., Andersson, S., Lawes, M.J. 2001. Sexual selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. Evolution 55(7): 1452-63

Pryke, S.R., Andersson, S., Lawes, M.J., Piper, S.E. 2002. Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. Behavioral Ecology 13(5): 622-631

Savalli, U.M. 1994a. Mate choice in the yellow-shouldered widowbird: correlates of male attractiveness. Behavioural Ecology and Sociobiology 35(4): 227-234

Savalli, U.M. 1994b. Tail length affects territory ownership in the yellow-shouldered widowbird. Animal Behaviour 48(1): 105-111

Savalli, U.M. 1995. Morphology, territoriality and mating system of the pintailed whydah *Vidua macroura*. Ostrich 66: 129-134

Sikkel, P.C. 1995. Effects of nest quality on male courtship and female spawning-site choice in an algal-nesting damselfish. Bulletin of Marine Science 57(3): 682-689

Skead, C.J. 1956. A study of the red bishop bird. Ostrich 27: 112-126

Svensson, O., Kvarnemo, C. 2003. Sexually selected nest-building – *Pomatoschistus minutus* males build smaller nest openings in the presence of sneaker males. Journal of Evolutionary Biology 16(5): 896-902

Verner, J., Engelsen, G.H. 1970. Territories, multiple nest-building, and polygyny in the long-billed marsh wren. Auk 87: 557-567

Woodall, P.F. 1971. Notes on a rhodesian colony of the red bishop. Ostrich 42: 205-210

Woodall, P.F. 1984. Nest height of the red bishop (*Euplectes orix*). South African Journal of Zoology 19: 261-265

Chapter 2

Behavioural time budgets and nest-building efficiency affect male reproductive success in Red Bishops (*Euplectes orix*)

1 Abstract

Red Bishop birds are a highly polygynous, colonially breeding species occurring in southern Africa with a high variation in male reproductive success. Male reproductive success is known to be determined mainly by the number of nests a male builds. This study investigates the influence of nest-building behaviour, courtship behaviour and male-male interactions on male nest-building performance, reproductive success, and territory tenure. For three different seasons behavioural patterns of males were analysed by collecting data on male time budgets. The importance of the number of nests built for reproductive success was confirmed. In addition, a higher number of nests built was related to more time spent with nest-building and more efficient nest-building, but nest-building efficiency could not compensate for less time spent with nest-building, the main determinant of nest-building performance. Neither the amount of time spent with courtship behaviour nor the amount of time spent with male-male interactions was related to reproductive success. Male reproductive success independent of the number of nests built was determined by the number of incorporated fibres, suggesting some importance of nest quality in terms of nest density for male reproductive success. There were no trade-offs with regard to time allocated to different behaviours. Instead, males differed in their general activity which might be due to differences in energy resources and may thus reflect male quality. Such differences in male quality could also be important for the amount of time allocated to nest-building and influence nest-building performance.

2 Introduction

Species with a polygynous mating system offer a good opportunity to study effects of sexual selection on reproductive success (e.g. Andersson 1982, Clutton-Brock & Vincent 1991, Cowlishaw & Dunbar 1991, Andersson *et al.* 2002). In polygynous mating systems, male reproductive success is often skewed with few males obtaining most matings and many males not mating at all. This high variation in reproductive success enhances sexual selection pressures on males (review in Andersson 1994) and facilitates the detection of male traits influencing reproductive success.

In some polygynous bird species, male reproductive success is mainly determined by the number of nests built by a male. In North American Long-billed Marsh Wrens (*Telmatodytes palustris*), male mating success depended only on the number of nest sites, and no other factors were found to influence male mating success (Verner & Engelson 1970). In European Wrens (*Troglodytes troglodytes*), male mating success depended on both the number of nests built and tail length (Evans & Burn 1996). Tail length probably plays a role in male-male competitions over territories in which nests survive better. Additionally, Evans (1997) suggested that the number of nests constructed by a male wren might depend on its building ability and physical condition. In African Yellow-shouldered Widowbirds (*Euplectes macrourus*) male mating success again depended only on the number of nests built by a male (Savalli 1994). In the polygynous and colonially breeding Red Bishop (*Euplectes orix*), male reproductive success is mainly determined by the number of nests a male builds in his territory during a breeding season (Friedl & Klump 1999, Lawes *et al.* 2002). Males establish and defend small territories in reed beds or bulrush stands around water, where they construct several nests to which they try to attract females. Nest frames are built exclusively by males, whereas females only line the inside of nests. Males provide no paternal care and their territories do not contain food or any other resources besides the nests. Apparently females settle randomly with regard to the number of nests within a territory (Friedl & Klump 2000, Lawes *et al.* 2002) and do not prefer males with a large number of nests at the time of the female's visit. Thus, there is strong sexual selection acting on male Red Bishops to build as many nests as possible without apparent female choice, since in a mating system with random female settlement male mating success will be directly related to the number of nests built (Friedl & Klump 2000, Lawes *et al.* 2002). The number of nests built by a male in turn depended on territory tenure, the number of nests built per week (Friedl & Klump 1999), and territory size (Lawes *et al.* 2002). Despite the importance of building many nests, considerable variation exists among territorial males with regard to the number of nests built during the course of a breeding season (Friedl & Klump 1999).

Here I investigate in more detail possible reasons for this high variation with regard to the number of nests built by territorial Red Bishop males. I present a detailed analysis of male behavioural patterns and nest-building efficiency to address the following questions: (1) Do males that construct more nests during the breeding season spend more time with nest-building, and if so, are there any trade-offs with other behavioural patterns? (2) Do males that construct more nests during the breeding season build more efficiently than other males, indicating better inherent or acquired nest-building ability? (3) Independent of the number of nests built by a male, do other aspects of nest-building behaviour influence male reproductive success? (4) In addition, I investigate whether other behavioural patterns such as the proportion of time spent with male-male interactions or courtship behaviour are related to male reproductive effort (number of nests built) or reproductive success (number of nests accepted).

3 Methods

3.1 Study site

Time budget patterns of male Red Bishops were studied during three breeding seasons (1998/1999, 2000/2001 and 2002/2003) at a colony in the Addo Elephant National Park in South Africa (33°26´ S, 25°45´ E). The study colony was situated at a small pond surrounded by bulrushes (*Typha capensis*) and common reeds (*Phragmites australis*). 52 males established a territory in the season 1998/1999, 50 males were territorial in 2000/2001, and 55 males were territorial in the season 2002/2003. All territorial males were in the typical bright red and black breeding plumage. During the period of this study, the number of nests built per season varied from 1 to 24 among all territorial males and mating success in terms of number of accepted nests per male varied from 0 to 20. Occasionally, one year old males in non-breeding plumage also built nests and showed courtship behaviour, but these nests were never accepted by females. For a description of the general biology and breeding system of Red Bishops, see Skead (1956), Emlen (1957), Craig (1974), and Friedl (2004).

3.2 General field methods

All nests of adult territorial males were individually marked with small tags attached to reeds close to the nest. Nests were controlled every other day to check the state of the nest, the number of eggs and the number of nestlings in the nests. For each nest I recorded the date on which nest-building started (defined as the first day on which a ring was visible), the date on which the nest was finished (defined as the day on which the breeding chamber was closed), and the date of acceptance (defined as the first day on which the nest contained one ore more eggs).

For every nest, the identity of the male building it was recorded. Birds were caught with mist nets and traps baited with commercially available mixed seeds. Each bird was ringed with a numbered metal ring obtained from the South African Bird Ringing Unit and four coloured plastic rings for individual identification. Some studies showed that rings with colours of secondary sexual traits are able to influence female choice (e.g. Burley *et al.* 1982, Metz & Weatherhead 1991). Therefore each individual was equipped with a red ring to level out any influence on female choice. In the study season 2002/2003, breeding chamber density of a total of 210 nests was measured by holding a piece of thin wire of 1cm length on the backside of the breeding chamber and counting the number of fibres going across this wire.

3.3 Time budget recordings

Time budgets were recorded at times of high activity, i.e. between 6:30 am and 9:00 am. Observations were continuous focal samples (Altmann 1974). During the seasons 2000/2001 and 2002/2003, 10 males constituted one observation block lasting 10 days and were observed each day for 10 min. To correct for a possible influence of time, the order in which males were observed was systematically rotated following a randomised block design. Time budgets could never be recorded on ten consecutive days because of failure of technical equipment or rain. In the season 1998/1999, 10 males were observed by Lars H. Hansen and Nicole Geberzahn 3 times a day for 3 min, a total of 9 min per day. During the season 2000/2001, time budgets of 10 males were recorded. During 2002/2003, time budgets of 20 males were recorded, in two blocks of observation separated by two months with 10 males each. The second block was excluded from the analysis because of insufficient breeding activity, with males being away from their territories most of the time. One male was observed in all three seasons and was excluded from the analysis for two randomly chosen seasons to guarantee independence of data when data for all three seasons were combined. Some males had to be excluded because they were not completely ringed and it could not be excluded that they were observed in at least one other season. Other males were excluded because of insufficient data points due to absence from their territory; this caused the dataset to be slightly biased towards above-average performing males. In the end 9 males entered the analysis for

1998/1999 and 2000/2001 and 6 males entered the analysis for 2002/2003 when data for all three seasons were combined. When each season was analysed separately, time budgets of 10 males were available for the seasons 1998/1999 and 2000/2001. For 2002/2003, time budgets of 9 males were analysed.

Recorded time budgets and behaviours were nest-building, fetching a reed fibre, fetching a bulrush fibre, stealing a fibre from another nest, leaf-stripping, threatening a neighbour, fighting a neighbour, threatening an intruder, fighting an intruder, chasing a conspecific in non-breeding plumage (female or yearling male), nest advertisement, copulation, swivelling, swaying, rattling, interaction with cape weavers (*Ploceus capensis*) or masked weavers (*Ploceus velatus*), present in territory. The time spent with each behaviour was recorded, except copulations, for which only occurrence was counted because they last only 1-2 seconds in Red Bishops. See Table 1 for definitions all recorded behaviours. In 1998/1999, the same behavioural patterns with the same definitions were recorded by Lars H. Hansen and Nicole Geberzahn with the exception of fetching a fibre.

Based on the time budget recordings I calculated the following behavioural parameters. Nest-building behaviour was described with (i) the proportion of time spent with nest-building and fetching fibres, (ii) the total number of fibres built into a nest per 10 min observation unit (in the following total number of fibres woven in; not for 1998/1999), and (iii) nest-building efficiency calculated as the total number of fibres built into a nest per 10 min observation unit divided by the time spent with nest-building (not for 1998/1999). Additionally, the proportion of time spent in territory (in the following territory attendance), the proportion of time spent with courtship behaviour including swivelling and swaying, and the proportion of time spent with male-male interactions were determined, including threatening of and fights with both neighbours and intruders. As courtship behaviour I considered only courtship behaviour directed at a specific female, i.e. nest advertisement, swivelling, swaying, and copulation and not non-directed courtship behaviour, i.e. rattling. All recorded behaviours were summarised as general activity. Sitting, hopping from one stem to another one, preening and sleeping were not recorded. Subtracting general activity from total time in territory did not yield something like doing nothing, because behaviours like hopping and preening are not equal to doing nothing. Instead, subtracting general activity from total time in territory would yield times of low activity.

Proportions were calculated relative to total observation time for times spent with each of the recorded behaviours.

Table 1 Recorded behaviours and their definitions.

general context	behaviour	definition
nest-building	nest-building	working on the nest, inside or outside
	fetching a reed fibre	tearing off a fibre from a leaf of common reed
	fetching a bulrush fibre	tearing off a fibre from a leaf of bulrush
	stealing a fibre from another nest	stealing a fibre from the nest of another male
	leaf-stripping	tearing off the tips of leaves of reeds or bulrush surrounding a nest
male-male interactions	threatening a neighbour	threat display (Craig 1974) directed at a neighbour
	fighting a neighbour	focus male and neighbour pecking each other
	threatening an intruder	threat display (Craig 1974) directed at an intruder
	fighting an intruder	focus male and intruder pecking each other
courtship	nest advertisement	male hanging below its nest while a female is inspecting the nest
	copulation	copulation
	swivelling	male swivelling around a reed or bulrush stem, at the same time singing (Craig 1974)
	swaying	male close to a female swaying back and forth with its body, singing (Craig 1974)
	rattling	perched on top of a reed or bulrush, rattling (Craig 1974)
other	chasing a conspecific in non-breeding plumage	females and yearling males could not be distinguished by sight and therefore combined
	present in territory	focus male is in its territory
	interaction with cape or masked weavers	threatening of or fighting with cape or masked weavers

3.4 Measures of male reproductive performance

To test whether the recorded time-budget patterns had significant effects on overall male reproductive performance, three different aspects of male performance were analysed. Reproductive effort was measured in terms of the number of nests built (defined as the number of completed nests per male for the whole season), and territory tenure (defined as the number of days from the beginning of the first nest until the last nest finished or accepted). Reproductive success was measured in terms of the residuals of the regression of the number of accepted nests against the number of nests built to eliminate the known strong effect of the number of nests built on reproductive success (Friedl & Klump 1999, Lawes *et al.* 2002, Chapter 1). For all three analysed seasons, the number of accepted nests was well predicted by the number of nests built (1998/1999: adjusted $R^2 = 0.687$, $p < 0.0005$; 2000/2001: adjusted $R^2 = 0.792$, $p < 0.0005$; 2002/2003: adjusted $R^2 = 0.530$, $p < 0.0005$).

4 Results

4.1 Analysed behavioural patterns

Table 2 Descriptive statistics of the analysed behavioural patterns. Values are percentage of time spent with each behavioural pattern, apart from the number of fibres, which represents the number of fibres woven in per observation unit, and efficiency, which represents the number of fibres woven in per min spent with nest-building.

Pattern	season	median	1. quartile	3. quartile	minimum	maximum
general activity %	1998/1999	8.0	6.7	10.5	1.7	18.1
	2000/2001	22.3	15.6	24.2	10.8	29.4
	2002/2003	17.9	15.4	25.3	12.3	30.1
nest-building %	1998/1999	2.6	1.1	6.9	0.0	11.1
	2000/2001	11.1	7.0	14.2	6.8	20.1
	2002/2003	10.9	10.5	13.9	5.5	16.2
courtship %	1998/1999	1.9	0.2	2.9	0.0	3.9
	2000/2001	1.8	1.4	2.4	0.9	4.7
	2002/2003	4.3	3.4	5.5	1.9	12.8
male-male interactions %	1998/1999	1.1	0.6	1.9	0.4	3.2
	2000/2001	1.4	0.5	2.2	0.0	2.7
	2002/2003	1.0	0.6	1.8	0.4	2.6
territory attendance %	1998/1999	76.8	45.1	93.7	35.7	98.9
	2000/2001	91.1	87.8	93.9	70.2	95.3
	2002/2003	88.2	83.2	93.6	66.7	97.8
n fibres	2000/2001	1.48	1.10	1.84	0.79	2.50
	2002/2003	1.60	1.40	2.46	1.14	3.00
efficiency	2000/2001	0.783	0.569	1.130	0.281	1.404
	2002/2003	0.802	0.595	1.361	0.284	1.710

Most of the time males were either sitting still or hopping around. Only a small proportion of time was spent with behaviour possibly related to reproductive success (see Table 2). Especially courtship behaviour was relatively rare, but here I only took directed courtship behaviour into account and neglected non-directed behaviour like singing.

4.2 Differences between seasons

Since intensity and course of breeding seasons can vary considerably, I tested for differences between the three analysed breeding seasons in regard to the number of nests built, the number of accepted nests, territory tenure, and time-budgets of all recorded behavioural patterns. For each season, means of the analysed behavioural patterns were calculated for each male.

Using a Kruskal-Wallis test, significant differences among seasons were detected with regard to the number of nests built (CHI-square = 8.09, df = 2, p = 0.017), the number of accepted nests (CHI-square = 8.06, df = 2, p = 0.018), time spent with nest-building (CHI-square = 12.59, df = 2, p = 0.002), time spent with courtship (CHI-square = 9.09, df = 2, p = 0.011), and general activity (CHI-square = 14.37, df = 2, p = 0.001) (see Figure 1). No significant differences among seasons were found in regard to territory tenure, territory attendance and time spent with male-male interactions (Kruskal-Wallis tests, all p > 0.1). There were no differences between 2000/2001 and 2002/2003 in regard to the total number of fibres woven in or nest-building efficiency (Mann-Whitney-U tests: both p > 0.4)

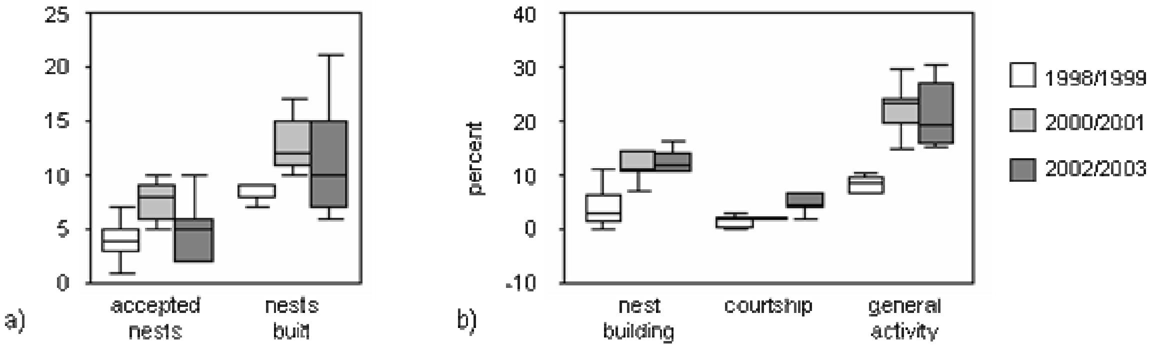


Figure 1 The three analysed seasons differed a) in the number of nests built and nests accepted per male and b) in the percentage of time males allocated to nest-building, courtship behaviour and general activity. Displayed are boxplots with median, quartiles and ranges.

4.3 Differences between males

In 1998/1999, males differed with respect to time spent with nest-building (Kruskal-Wallis: Chi-square = 18.70, df = 9, p = 0.028), territory attendance (Kruskal-

Wallis: Chi-square = 24.44, df = 9, p = 0.004), and general activity (Kruskal-Wallis: Chi-square = 19.10, df = 9, p = 0.024), while no significant differences were found with regard to the time spent with male-male interactions or with courtship (Kruskal-Wallis: both p > 0.2). For 2000/2001, males differed significantly only with regard to nest-building efficiency (Kruskal-Wallis: Chi-square = 21.79, df = 10, p = 0.016). For 2002/2003 no significant differences between males were found with regard to any of the behavioural parameters investigated (Kruskal-Wallis tests: all p > 0.1).

To test for differences between males with data from more than one season combined, data were z-transformed for each season separately to make seasons comparable. All three seasons combined, males differed in the time spent with courtship (Kruskal-Wallis: Chi-square = 36.24, df = 23, p = 0.039), in territory attendance (Kruskal-Wallis: Chi-square = 74.56, df = 23, p < 0.0005), and in their general activity (Kruskal-Wallis: Chi-square = 40.15, df = 23, p = 0.015), but not in the time spent with nest-building (Kruskal-Wallis: Chi-square = 26.64, df = 23, p = 0.27) and not in the time spent with male-male interactions (Kruskal-Wallis: Chi-square = 34.86, df = 23, p = 0.054).

When data were combined for the seasons 2000/2001 and 2002/2003 only, there were significant differences between males in the total number of fibres woven in (Kruskal-Wallis: Chi-square = 30.79, df = 14, p = 0.006) and in nest-building efficiency (Kruskal-Wallis: Chi-square = 33.36, df = 19, p = 0.022). Both of these variables were only recorded in the seasons 2000/2001 and 2002/2003.

4.4 Correlations between behavioural patterns

To test if there was a trade-off between different behavioural patterns with regard to the time invested in each pattern I first divided time spent with nest-building, time spent with male-male-interactions and time spent with courtship through territory attendance to obtain proportions for times spent with recorded behaviours while in territory. These proportions were z-transformed for each season separately, before data were pooled for all three seasons. Bonferroni corrections (Sokal & Rohlf 1995) were applied to adjust the level of significance for all multiple tests using the same variables. In case of trade-offs, there should have been negative correlations between time invested in different behavioural patterns, but no such significant negative correlations were found (Table 3). This suggests that instead of trade-offs among different activities there were differences among males in regard to overall activity and thus confirms the finding reported above. General activity correlated positively with time spent with nest-building (Table 3). As can be seen in Table 2, more time was allocated to nest-building than to other behaviours possibly related to reproductive success and therefore time spent with nest-building contributed most to general activity.

Table 3 Correlations between analysed patterns. Data were pooled over the three analysed seasons. N = 24

		nest-building	male-male interactions	courtship	general activity
territory	r	0.137	-0.359	-0.072	0.389
attendance	p	0.523	0.085	0.738	0.060
nest-	r		-0.251	0.195	0.821
building	p		0.238	0.361	< 0.0005
male-male	r			0.005	-0.227
interactions	p			0.982	0.286
courtship	r				0.497
	p				0.013

When data for 2001/2002 and 2002/2003 were combined, time spent with nest-building correlated significantly with the total number of fibres woven in (N = 15, r = 0.76, p = 0.001). In addition, there was a significant correlation between general activity and time spent with nest-building (N = 15, r = 0.90, p < 0.0005). The total number of fibres woven in can be explained by the time spent with nest-building combined with nest-building efficiency. To test this assumption I calculated a stepwise multiple regression with the total number of fibres woven in as dependent variable and time spent with nest-building and nest-building efficiency as independent variables. Both variables together explained the total number of fibres woven in (adjusted $R^2 = 0.65$, $F_{2,14} = 14.08$, p = 0.001), but time spent nest-building (T = 4.48, p = 0.001, partial r = 0.79) was more important than nest-building efficiency (T = 2.24, p = 0.045, partial r = 0.54). When data for each season were analysed separately, one significant correlation was found after Bonferroni corrections were applied. In 2000/2001 general activity was positively correlated to time spent with nest-building (N = 10, r = 0.92, p < 0.001).

4.5 Behavioural patterns and male performance

To investigate the influence of the recorded behavioural patterns on male reproductive performance I performed stepwise multiple regression analyses with male reproductive effort (number of nests built), territory tenure (number of consecutive days holding a territory), or reproductive success (residuals of the regression of the number of nests accepted against the number of nests built) as dependent variable. The behavioural patterns used as independent variables in the multiple regression models presented in the following sections were time spent with nest-building, time spent with male-male interactions, time spent with courtship, territory attendance, and general activity. For the analyses involving only the seasons 2000/2001 and 2002/2003, the total number of fibres woven in and nest-building efficiency were additionally available as independent variables.

4.5.1 Reproductive effort

The influence of the analysed behavioural parameters on reproductive effort measured as the number of nests built was investigated for all three seasons combined, the seasons 2000/2001 and 2002/2003 combined, and each season separately. For an overview of determinants of reproductive effort, see Table 4.

When data for all three study seasons were combined, a stepwise multiple regression with the number of nests built as dependent variable and the behavioural parameters as independent variables revealed a significant final model (adjusted r^2 = 0.15, $F_{1,23}$ = 4.99, p = 0.036) which included only time spent with nest-building (T = 2.47, p = 0.022, r = 0.47). Since in 1998/1999 data on the total number of fibres woven in and nest-building efficiency were not recorded, I performed another stepwise regression combining data from the seasons 2000/2001 and 2002/2003 only. The resulting final model (adjusted r^2 = 0.65, $F_{2,14}$ = 14.05, p = 0.001) included the time spent with nest-building (T = 4.61, p = 0.001, partial r = 0.80) and nest-building efficiency (T = 2.75, p = 0.018; partial r = 0.62).

When each season was tested separately, no significant effect of any of the behavioural parameters on the number of nests built was found in 1998/1999 and 2002/2003. For 2000/2001 the stepwise regression resulted in a final model that again included only the time spent with nest-building (adjusted r^2 = 0.64, $F_{1,9}$ = 16.76, p = 0.003).

Table 4 Results of stepwise multiple regressions with the number of nests built by a male (reproductive effort) as dependent variable. Only behavioural patterns with a significant influence on reproductive effort are displayed.

All three seasons combined	2000/2001 + 2002/2003	1998/1999	2000/2001	2002/2003
time spent with nest-building	time spent with nest-building nest-building efficiency	–	time spent with nest-building	–

4.5.2 Territory tenure

When territory tenure was used as dependent variable, there was no significant final model of stepwise multiple regressions for all three seasons combined, the two seasons 2000/2001 and 2002/2003 combined and the season 1998/1999. In 2000/2001, territory tenure was predicted by territory attendance (adjusted $r^2 = 0.66$, $F_{1,9} = 18.65$, $p = 0.003$). In 2002/2003, territory tenure was predicted by nest-building efficiency (adjusted $r^2 = 0.45$, $F_{1,9} = 7.51$, $p = 0.029$).

4.5.3 Reproductive success

Since I wanted to investigate male mating success independently of the number of nests built (see 2.4), I used the residuals of the regression of the number of nests accepted against the number of nests built as measure for reproductive success. For an overview of determinants of reproductive success, see Table 5. When data were combined for the three study seasons, a multiple regression with the residuals of the regression of the number of accepted nests against number of nests built as dependent variable showed no significant effect of any of the behavioural patterns investigated. A stepwise regression for 2000/2001 and 2002/2003 with the total number of fibres woven in and nest-building efficiency added as independent variables resulted in a final model (adjusted $r^2 = 0.31$, $F_{1,14} = 7.47$, $p = 0.017$) that included only the total number of fibres woven in ($T = 2.73$, $p = 0.017$, $r = 0.60$). The higher the total number of fibres woven in, the higher was a males' reproductive success (see Figure 2).

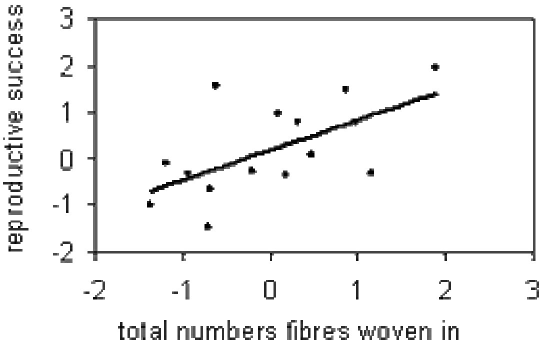


Figure 2 Reproductive success correlated with the total number of fibres woven in. Displayed are data for seasons 2000/2001 and 2002/2003 combined; values are z-transformed for each season separately.

When stepwise regressions with reproductive success as dependent variable were performed for each season separately, no significant effects of any of the behavioural parameters investigated were found in 1998/1999. The final model for the season 2000/2001 included only the total number of fibres woven in (adjusted $r^2 = 0.49$, $F_{1,9} = 9.63$, $p = 0.015$), while the final model for the season 2002/2003 included only territory attendance (adjusted $r^2 = 0.37$, $F_{1,8} = 5.65$, $p = 0.049$).

Table 5 Results of stepwise multiple regressions with reproductive success as dependent variable. Only behavioural patterns with a significant influence on reproductive success are displayed.

all three seasons combined	2000/2001 + 2002/2003	1998/1999	2000/2001	2002/2003
–	total number of fibres woven in	–	total number of fibres woven in	territory attendance

An analysis of nest characteristics showed that more densely woven nests had a higher likelihood of becoming accepted. To test the hypothesis that nests of males who incorporate more fibres are more densely woven, correlations were calculated between the number of fibres woven in and density of nests built by a male. Average nest density of a male and the average number of fibres woven in per observation unit did not correlate ($r_s = 0.306$, $N = 16$, $p = 0.249$).

5 Discussion

Earlier studies by Friedl & Klump (1999) and Lawes *et al.* (2002) showed that the number of nests built is the strongest determinant of reproductive success in male Red Bishops. The number of nests built, termed reproductive effort in this study, was in turn predicted by the number of weeks a male held a territory (territory tenure) and the number of nests built per week (Friedl & Klump 1999). In the study by Lawes *et al.* (2002), only territory size had a significant positive effect on the number of nests built (cock's nests). Determinants of male reproductive success were also investigated in a number of other *Euplectes* species. Previous studies demonstrated female choice for male ornaments, especially tail length in *E. progne* (Andersson 1982), *E. jacksonii* (Andersson 1989), *E. ardens* (Andersson *et al.* 2002), and *E. axillaris* (Pryke & Andersson 2002). The number of nests built best predicted mating success in *E. macrourus* (Savalli 1994), whereas male morphology was not found to be related to mating success. Possible mechanisms leading to differences in the number of nests built by males were not investigated for *E. macrourus*. Here I present a detailed analysis investigating the influence of male time-budget and behavioural patterns on reproductive performance as assessed by the number of nests built, territory tenure, and reproductive success (independent of the effect of the number of nests built). This analysis aims to exceed earlier studies by focussing on the importance of nest-building behaviour and efficiency on reproductive performance.

5.1 Reproductive effort

Reproductive effort in terms of the number of nests built was higher for males that spent more time with nest-building and increased further with higher nest-building efficiency. Lawes *et al.* (2002) investigated the influence of time in territory, sexual activity, territory size, nest-building, number of movements, and time spent conspicuous on the number of nests a male built. Only territory size had an effect on the number of nests built in their study, not time spent with nest-building. In this study, males that constructed more nests during the breeding season also built more efficiently (data combined for the two seasons for which nest-building efficiency was recorded). Sometimes males work on a nest without incorporating a new fibre, but rearrange and correct the existing frame. Such behaviour should decrease nest-building efficiency. A high nest-building efficiency, here defined as the ability to incorporate many fibres per time spent with nest-building, implies that only few corrections on the nest frame are necessary and during most of the time spent with nest-building a new fibre is incorporated.

5.2 Territoriality and fighting ability

Friedl & Klump (1999) also found territory tenure to determine the number of nests built by a male. Territory tenure in turn might depend on the frequency of male-male interactions and the proportion of time spent in the territory (territory attendance). However, none of these two parameters investigated in this study was found to consistently influence territory tenure. The influence of male-male interactions on territory tenure is apparently not based on the quantity of these interactions. Instead, a qualitative assessment of male-male interactions might better reflect their influence on territory tenure. Unfortunately most interactions between neighbouring territorial males did not end with a clear winner and a clear loser, both males rather retreated simultaneously. The few interactions that did allow for a clear assignment of winner and loser were not sufficient in number to allow statistical analysis. Interactions between a territorial male and an intruder always followed a stereotypical pattern with the intruder being chased away immediately (Skead 1956, Craig 1974).

A male Red Bishop needs a territory within which nests are built. Therefore the establishment of a territory is crucial for male reproductive success in Red Bishops. The amount of fighting could well play a role in the establishment of a territory in male Red Bishops, as is the case in lekking Black Grouse (*Tetrao tetrix*). Male Black Grouse that were more often involved in fights had larger territories and higher mating success (Höglund *et al.* 1997). During high breeding activity in a Red Bishop colony, agonistic interactions between neighbouring territorial males are frequently observed (Craig 1974, Friedl 2004) and an abandoned territory is quickly taken over by other males. Although Höglund *et al.* (1997) stated that successful males seemed to be more active, in the presence of females they showed less rookooing, a vocal display, and no difference in circling, a courtship behaviour. These two displays, together with fighting, are the only ones shown in the presence of females. Such differences between males as for Black Grouse in time budgets of courtship behaviour and male-male interactions relative to mating success were not observed in Red Bishops.

5.3 Reproductive success

Reproductive success independent of the number of nests built was predicted only by the total number of fibres woven in. In other words, of two males with an equal number of nests built, the one incorporating more fibres profited from higher reproductive success. The total number of fibres woven in could be explained by the time spent with nest-building in combination with nest-building efficiency. The latter two also explained the number of nests built by a male, but in the measure of reproductive success used here, the number of nests built was controlled for. Because the effect of number of nests built was eliminated, and the number of nests built was mainly predicted by the time spent with nest-building, it can be excluded that the effect of the total number of fibres woven in on reproductive success is merely due to more time allocated to nest-building, reflecting higher levels of general activity of healthier and fitter males. Instead, the total number of fibres influenced reproductive success independent of the number of nests built, suggesting that some aspect of nest quality related to the total number of fibres woven in (such as nest density) might contribute to male reproductive success. The fact that females preferred more densely woven nests (see Chapter 1) strengthens this assumption, but average density of nests built by a male did not correlate with the average number of fibres woven in per observation unit. This could be a problem of the small sample size.

5.4 Time budgets

The number of nests built was predicted by the time spent with nest-building, a behaviour in which males invested up to 20% of their time. This large amount of time spent with one activity could lead to the assumption of trade-offs in time allocated to different behavioural patterns. However, there were no significant negative correlations between time spent with nest-building, time spent with male-male interactions and time spent with courtship, indicating that time allocated to one activity does not necessarily constrain other activities, as is often found for parental effort and additional mating effort (Cucco & Malacarne 1997, Peters 2002, Margrath & Komdeur 2003). Energetic costs of nest-building could pose a constraint for males of other species of weaverbirds, as Collias & Collias (1984) hypothesized based on observations and calculations of energy demands for nest-building. Collecting fibres demands high energetic costs because it involves flight. However, considering the short distances covered to collect fibres from bulrush and reed at the colony site (5-20m, pers. obs.), energy expenditure for nest-building is probably rather small and therefore not a serious constraint for male Red Bishops. Instead of trade-offs between behavioural patterns, there were significant differences between males with regard to their general activity. It remains to be explained why some males allocated more time to nest-building and general activity. There was a tendency of males spending more time in their territory also showing more activity while they were in their territory. This could reflect better foraging abilities (less time spent away from the territory for feeding), and better body condition (higher activity levels while on the territory). Since high activity levels while on the territory together with a lot of time spent in the territory pose strain on energetic budgets because only little time is spent away from the territory with foraging, these differences might ultimately reflect differences in individual male quality. Such differences in foraging ability and body condition could explain differences in time budgets between males, as observed in Black Redstarts (*Phoenicurus ochrurus*) with artificial food-supply (Cucco & Malacarne 1997).

In a lekking sandpiper, the ruff (*Philomachus pugnax*), lekking males with higher activity levels that spend more time on the lek territory had higher reproductive success (Bachman & Widemo 1999). Differences between males in their time allocation to certain activities relevant for reproductive success are also apparent in a monogamous moorhen (*Gallinula chloropus*; Petrie 1983). In this species, a pair can initiate and raise several clutches per breeding season, and males perform most of the incubation. Since incubation is energetically expensive, male moorhens face considerable energetic constrains, and higher quality males can incubate for longer periods. In addition, high quality males are scarce in this species, and females compete for such males, i.e. small males with large fat reserves. These small fat males can maintain time budgets with long periods of incubation and thus females paired to small fat males could initiate more nesting attempts during a breeding season. Similarly, higher activity levels might well reflect individual quality in male Red Bishops.

A study on house sparrows (*Passer domesticus*) by Václav *et al.* (2003) showed how differences in time budgets of females can substantially change reproductive success of males. At nest sites with higher food supply female house sparrows spent more time at their nest site, and the proportion of extra-pair paternity was lower. Males did not change nest site attendance or within-pair copulation frequency in relation to food supply. In food-rich habitats, females were less exposed to forced copulations by other males, thus reducing the proportion of extra-pair paternity in their clutches as a result of changed time budgets.

In Superb Fairy-wrens (*Malurus cyaneus*), males treated with testosterone changed their time budgets and invested more time in courtship and less time in parental care (Peters 2002). This shift could increase within-pair as well as extra-pair mating success in Superb Fairy-wrens, but decrease reproductive success if offspring receives less parental care. Interestingly, testosterone treated males never showed the maximum possible amount of extra-pair courtship behaviour when nestlings required parental care. To achieve high reproductive success, a compromise between different behaviours must be found in male Superb Fairy-wrens. Such a trade-off between different behaviours was absent in male Red Bishops, where nest-building behaviour did not impose constrains on courtship behaviour.

Adverse conditions can affect energy expenditure rates and time budgets, as shown for a hummingbird, the Green-backed Firecrown (*Sephanoides sephanoides*), whose activity levels dropped with ambient temperature to reduce body mass loss (López-Calleja & Bozinovic 2003). Higher quality individuals coping better with such adverse conditions could maintain higher levels of activity and thus gain an advantage over other individuals. For Red Bishops living in semi-arid subtropics, it would be rather high than low temperatures during breeding season that constrain times of activity. On hot days, general activity in the observed colony declined markedly from 11am on and only resumed in the evening hours (pers. obs). Thus it is conceivable that males more tolerant to high temperature could allocate more time to nest-building and thus enhance reproductive success. This hypothesis could be tested in further studies on male quality and performance.

Other behavioural patterns than nest-building analysed in this study were male-male interactions and time spent with courtship. Neither time spent with male-male interactions nor time spent with courtship were related to the number of nests built or reproductive success independent of the number of nests built. In one season, the percentage of time a male spent in its territory had an effect on mating success independent of the number of nests built. Male Red Bishops show conspicuous and elaborate courtship behaviour (Emlen 1957, Craig 1974) when a female perches close to them. Males also show this directed courtship display when the female is in a neighbour's territory. In the study colony with rather small territories, a perching female surrounded by several males engaged in courtship display was a common sight. The female would follow one male, inspect a nest and sometimes allow the male to copulate (pers. obs.). Thus if a male spends a lot of time outside its territory it might miss on mating opportunities and experience lower reproductive success compared to a male with an equal number of nests that spends more time in the territory.

5.5 Differences in time budgets between seasons

The three analysed season differed in the general breeding activity shown by males and females. Breeding intensity depends on food supply, which depends on rainfall patterns. Rainfall is known to determine the timing and length of a breeding season in arid and semi-arid zone birds (Brooke 1966, Craig 1982, Lloyd 1999, Friedl 2002) and can lead to the observed differences in the mean number of nests built by males and accepted by females in different years (see Chapter 3). The observation that time spent with nest-building by males was lower in the first analysed season is probably due to the time during the breeding season when time budgets were recorded. Breeding activity was somewhat lower in the first season than in the other two seasons, and this is probably reflected in less time spent with nest-building. The period when time budgets were recorded is probably also explaining the difference in the amount of observed courtship behaviour. In the last season, time budgets were recorded when breeding activity was at its highest, whereas in the other two seasons time budget recordings took place at periods of high as well as lower breeding activity.

5.6 Conclusion

More parameters, detailed male behaviours and characteristics of territories like size, tenure time, and habitat must be analysed together to better understand the underlying determinants of male reproductive success and the effect of intrasexual selection in Red Bishop birds. An indicator for male physical condition independent of behavioural observations could substantially contribute to a better understanding of the breeding system of Red Bishops. Unfortunately, morphological data that could provide such an indicator, were not available for the observed male Red Bishops. The function and effect of the conspicuous male breeding plumage in male-male interactions and of the elaborate courtship displays remains unknown and should be addressed in future studies.

6 References

Altmann, J. 1974. Observational study of behaviour: sampling methods. Behaviour 49: 227-267

Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. Nature 299 (5886): 818-820

Andersson, S. 1989. Sexual selection and cues for female choice in leks of Jackson’s widowbird Euplectes jacksonii. Behav. Ecol. Sociobiol. 25(6): 403-410

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ

Andersson, S., Pryke, S.R., Ornborg, J., Lawes, M.J., Andersson, M. 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. Am. Nat. 160 (5): 683-691

Bachman, G., Widemo, F. 1999. Relationships between body composition, body size and alternative reproductive tactics in a lekking sandpiper, the Ruff (*Philomachus pugnax*). Functional Ecology 13: 411-416

Brooke, R.K. 1966. Nuptial moult, breeding season and clutch size of rhodesian Red Bishops *Euplectes orix* and congeners in relation to rainfall. Ostrich sup. 6: 223-235

Burley, N.T., Krantzberg, G., Radman, P. 1982. Influence of colour-banding on the conspecific preferences of zebra finches. Anim. Behav. 30: 444-455

Clutton-Brock, T.H., Vincent, A.C.J. 1991. Sexual selection and the potential reproductive rates of males and females. Nature 351(6321): 58-60

Collias, N.E., Collias, E.C. 1984. Nest Building and bird behavior. Princeton University Press, Princeton, N.J.

Cowlishaw, G., Dunbar, R.I.M. 1991. Dominance rank and mating success in male primates. Anim. Behav. 41: 1045-1056

Craig, A.J.F.K. 1974. Reproductive behaviour of the male Red Bishop bird. Ostrich 45: 149-160

Craig, A.J.F.K. 1982. The breeding season of the Red Bishop. Ostrich 53: 112-114

Cucco M, Malacarne G. 1997. The effect of supplemental food on time budget and body condition in the Black Redstart *Phoenicurus ochrurus*. Ardea 85(2): 211-221

Emlen, J.T.Jr. 1957. Display and mate selection in the whydahs and bishop birds. Ostrich 28: 202-214

Evans, M. R. 1997. Nest building signals male condition rather than age in wrens. Anim. Behav. 53: 749-755

Evans, M. R., Burn, J.L. 1996. An experimental analysis of mate choice in the wren: a monomorphic, polygynous passerine. Behavioral Ecology 7(1): 101-108

Friedl, T.W.P. 2002. The effect of rainfall on the breeding behaviour of the Red Bishop, *Euplectes orix*. Ostrich 73(3-4): 181-184

Friedl, T.W.P. 2004. Breeding behaviour of the Red Bishop (*Euplectes orix*): a synthesis and new observations. Die Vogelwarte 42: 177-190

Friedl, T.W.P., Klump, G.M. 1999. Determinants of male mating success in the Red Bishop (*Euplectes orix*). Behav. Ecol. Sociobiol. 46: 387-399

Höglund, J., Johannson, T., Pelabon, C., 1997. Behaviourally mediated sexual selection: characteristics of successful male black grouse. Anim. Behav. 54: 255-264

Lawes, M., Slotow, R., Andersson, S. 2002. Male nest building but not display behaviour directly influences mating success in the polygynous Red Bishop, *Euplectes orix*. Ostrich 73(3-4): 87-91

Lloyd, P. 1999. Rainfall as a breeding stimulus and clutch size determinant in South African arid-zone birds. Ibis 141: 637-643

López-Calleja, M.V., Bozinovic, F. 2003. Dynamic energy and time budgets in hummingbirds: a study in Sephanoides sephanoides. Comparative Biochemistry and Physiology Part A 134: 283-295

Magrath, M.J.L., Komdeur, J. 2003. Is male care compromised by additional mating opportunity? Trends in Ecology and Evolution 18(8): 424-430

Metz, K.J., Weatherhead, P.J. 1991. Color bands function as secondary sexual traits in male red-winged blackbirds. Behav. Ecol. Sociobiol. 28: 23-27

Peters, A. 2002. Testosterone and the trade-off between mating and paternal effort in extrapair-mating superb fairy-wrens. Anim. Behav. 64: 103-112

Petrie, M. 1983. Female moorhens compete for small fat males. Science 220: 413-415

Pryke, S.R., Andersson, S. 2002. A generalized female bias for long tails in a short-tailed widowbird. P Roy Soc Lond B Bio 269(1505): 2141-2146

Savalli, U.M. 1994. Mate choice in the yellow-shouldered widowbird: correlates of male attractiveness. Behavioural Ecology and Sociobiology 35(4): 227-234

Skead, C.J. 1956. A study of the Red Bishop bird. Ostrich 27: 112-126

Václav, R., Hoi, H., Blomqvist, D. 2003. Food supplementation affects extrapair paternity in house sparrows. Behavioral Ecology 14(5): 730-735

Verner, J., Engelsen, G.H. 1970. Territories, multiple nest building, and polygyny in the long-billed marsh wren. Auk 87: 557-567

Zur Homepage der Dissertation

Chapter 3

Temporal changes in demand for and supply of nests in Red Bishops (*Euplectes orix*): dynamics of a biological market

1 Abstract

Reproductive success in male Red Bishops depends mainly on the number of nests built by a male. In Red Bishops as in other colonially breeding, polygynous weaverbirds, nest frames are built by males alone and offspring is raised by females only. Thus in the mating system of Red Bishops, nests can be regarded as a commodity traded in a biological market. The characteristics of the Red Bishop’s mating system as a biological market for nests are described, as well as temporal changes and synchronisation of male and female breeding activity. Timing of breeding by birds in arid and semi-arid zones depends on rainfall patterns which are often unpredictable. This leads to temporal changes in demand for and supply of nests within a breeding season in Red Bishops. These temporal changes are enhanced by a female preference for young, fresh nests that demands of males to quickly adjust building activity. Breeding activity of males and females are highly synchronised. An increase in male nest-building activity is accompanied by an increase in the number of nests accepted by females in the same and the following week, matching the preference for young nests. Males increase their building speed with increasing female breeding activity, but males with a better adjustment of building speed to female breeding activity did not always gain higher mating success. The applicability of biological market theory to the Red Bishop’s mating system and the dynamics of this biological market are discussed.

2 Introduction

2.1 Biological markets

Many biological systems can be perceived as markets with an exchange of commodities, analogous to human markets (Noë & Hammerstein 1994). Such biological markets consist of distinct trading classes, e.g. pollinators and pollinated plants (Pellmyr & Huth 1994), breeders and helpers (Reyer 1986), or males and females (Stopka & Macdonald 1999, Dunbar 2001), that exchange goods and services to their mutual benefit (Noë & Hammerstein 1995). With regard to mating systems, such commodities and services can be gametes, parental care, nuptial gifts, good genes, or nests. An inherent feature of mating systems is the presence of two distinct classes, which makes mating systems particularly suitable for a biological market theory approach (Noë & Hammerstein 1994). The relation between two trading classes is often asymmetrical in the sense that members of one class can always find a trading partner in the other class and are choosing a member of the other class with its commodity offered (Noë & Hammerstein 1994). Members of the other class in turn have to compete between themselves in order to be accepted by a member of the choosing class. Conditions of a working market system are that commodities can not be obtained without the consent of the trading partner, and members of the same class competing for the same trading partner can not eliminate each other by force (Noë & Hammerstein 1994). In contrast to classic game theory models like the prisoner’s dilemma and its variations (Olstrom 2001) that look at interacting individuals, in market models two or more trading classes interact (Noë & Hammerstein 1994, 1995). The members of a trading class can choose and switch partners. Cheating is either not possible or effectively controlled for. Because of these fundamentally different approaches between game theories describing social dilemmas and market models, they are not competing with each other, but address different problems and can complement each other (Bshary 2001). Biological market theory was applied to several species of primates (e.g. Colmenares *et al.* 2002, Henzi & Barrett 2002, Eckardt & Zuberbühler 2004), initiated by Seyfarth’s model of grooming for support (Seyfarth 1977). Another example for a biological market model is the interspecific interaction between cleaner fishes and their clients (e.g. Bshary 2001, Bshary & Grutter 2002).

2.2 Nests as commodities in a market

In Red Bishops (*Euplectes orix*), a colonially breeding, highly polygynous weaverbird species occurring in southern Africa (McLean 1993), males and females can be regarded as two classes of traders. Commodities and services of males are nests, nest-building and gametes, commodities and services of females are gametes and the raising of offspring. In Red Bishops, nest frames are built by males alone, whereas females incubate eggs and feed nestlings without male assistance (Skead 1956, Emlen 1957). Therefore nests built by male Red Bishops can be regarded as commodities in a biological market, because they represent a commodity produced by one sex only, and they can be chosen by females as a trading class. Sampling costs for females are low, because territories of males are immediately adjacent in a breeding colony. Nests are easy to interpret in their function for both trading classes, contrary to e.g. grooming in primates, where benefits are context-dependent (Colmenares *et al.* 2002). The exchange value of a commodity in a market is determined by supply and demand and can vary over time. Such variation over time in demand for and supply of nests will be the focus of this study. Changes in breeding activity during a season shall be investigated, as well as demands on male nest-building behaviour and how males can behaviourally adjust to changing demand for nests.

2.3 Variation in demand and supply

Changes in breeding activity during a season are to be expected, because the timing of breeding can be of crucial importance for offspring survival. Sufficient food supply is important for survival and early development of offspring (e.g. Nowicki *et al.* 1998). Especially in semi-arid zones, rainfall is patchy in time as in space (Lovegrove 1993), and birds can react quickly to rainfall and start or enhance breeding activity (Brooke 1966, Collias & Collias 1978, Lloyd 1999, Friedl 2002). In Red Bishops, a typical breeding season lasts about five months with two distinct breeding peaks (Friedl 2002) and is linked to rainfall patterns over the whole range of occurrence (Craig 1982). These short-term peaks in breeding activity can occur any time during the breeding season. Sufficient food to raise young is often only available after rains and an important factor determining the timing of breeding in birds (Lloyd 1999). Sometimes birds even desert a breeding colony, probably due to limited food supply at the end of the breeding season, as is reported for Red Bishops (Skead 1956). Timing of breeding seasons of birds in southern African arid and semi-arid zones in relation to rainfall has been well studied (Brooke 1966, Craig 1982, Lloyd 1999, Friedl 2002). Within season rainfall patterns determine the onset and duration of a breeding season. In Red Bishops, poor rainfall prior to a breeding season can delay the start of a breeding season, and poor mid-season rainfall causes a breeding season to end earlier (Brooke 1966, Friedl 2002). On a smaller time scale, the number of nests built and the number of eggs laid within 10 days were related to the amount of rainfall 10-20 days ago (Friedl 2002). In the same study, the total amount of rainfall did not influence the duration of a breeding season, but average clutch size, as was found for the same species by Brooke (1966) and for a number of other bird species by Lloyd (1999).

2.4 Determinants of the exchange value of nests

Female Red Bishops not only need sufficient food supply after good rains for a successful breeding attempt, they also require a suitable nest. Consequently females should consider rainfall and food abundance in the area together with the supply of suitable nests before starting a breeding attempt. There are indications that male bishops and whydahs start building nest frames as soon as suitable building material in the form of fresh grass is available, whereas females only start laying eggs after rainfall sufficient enough to guarantee food supply needed to raise offspring (Brooke 1966). In such polygynous species, it can be expected that males try to attract as many females as possible and are therefore continuously building nests, at the same time adjusting their nest-building activity more to current female demand and less to rainfall. For a nest to become accepted it might not be sufficient to be merely available, instead some other characteristics inherent to nests might as well play a role. In Village Weavers (*Ploceus cucullatus*), young, green nests are much more advertised by males and accepted by females than old, brown nests (Collias & Victoria 1978). A similar preference in other weaverbirds can be expected since weaverbird nests are generally constructed with grass and reed fibres (Collias & Collias 1984), and was found for Red Bishops, where accepted nests were below the median age of all available nests, summarised over all days in a season on which nest were accepted (Hansen 2001). Such a preference for fresh nests in combination with a rapid increase of female breeding activity after heavy rainfall demands from males to build many nests in a short time in response to rainfall or, more directly, in response to many females searching for a suitable nest to start breeding.

2.5 The aim of this study

This study investigates the role of nests built by males as a commodity in a mating market. For this commodity, changes in demand and supply within a breeding season are described. To achieve this objective, timing of breeding in Red Bishops is analysed on a fine time scale, weeks. The exchange value of nests as a commodity depends on their age as a trait influencing the acceptance probability of a nest. Therefore the observed preference for young nests (Hansen 2001) is analysed in more detail, and its implications for male nest-building activity are investigated. Synchronisation of male and female breeding activity is analysed, as well as temporal relations between rainfall, the number of nests built by males and the number of nests accepted by females. In a system with more supply of a commodity than demand for it, it could be expected that supply is following demand and not vice versa. Further on, possible short-term adjustments of male supply in terms of nest-building speed to increasing female demand for nests and its effect on male mating success is investigated.

3 Methods

3.1 Observation of the Red Bishop colony in South Africa

3.1.1 Observed colony

The study colony of Red Bishops was located in the Addo Elephant National Park in South Africa in the Eastern Cape Province (33°26´ S, 25°45´ O), and three consecutive breeding seasons (2000/2001, 2001/2002 and 2002/2003) were analysed. Rainfall data were collected close to the colony and provided by South African Weather Services. Breeding seasons usually last from October until March with two distinct breeding peaks. The colony was situated at a small pond with nests suspended in surrounding reeds (*Phragmites australis*) or bulrushes (*Typha capensis*). 50 males established a territory in the season 2000/2001, compared to 64 males in the season 2001/2002 and 55 males in the season 2002/2003. All territorial males were in the typical bright red and black breeding plumage. One-year-old males in non-breeding plumage also built nests and showed courtship behaviour, but these nests were not accepted by females.

3.1.2 Monitoring of breeding activity

All nests of adult territorial males were individually marked with small tags attached to reeds close to the nest. Nests were controlled every other day to check for the state of the nest, number of eggs and nestlings in nests and to mark new nests. For each nest I recorded the date the male started building it (entrance ring detectable), the date of finishing (breeding chamber closed), the date of acceptance (occurrence of first egg), and the date on which it became unusable. A nest was regarded unusable when it was partially or completely destroyed, for example by Cape Weavers (*Ploceus capensis*) or Masked Weavers (*Ploceus velatus*) that often removed a Red Bishop nest to build their own nest in that place. A nest could also be rendered unusable when it was deformed to a degree that it was no longer possible for a bird to enter it because of a compressed entrance or an indented breeding chamber. Since most nests were accepted within two weeks after they were finished, the number of unusable nests comprised only nests that became unusable within two weeks after they were finished. With observations I recorded the identity of the male building a given nest and, if it was accepted, tried to identify by which female it was accepted. Birds were caught with mist nets and traps baited with commercially available mixed seeds. Each bird was ringed with a numbered metal ring obtained from the South African Bird Ringing Unit and four coloured plastic rings for individual visual identification. Previous studies showed that rings with colours similar to secondary sexual traits are able to influence male and female mating behaviour (e.g. Burley *et al.* 1982, Metz & Weatherhead 1991, Johnson *et al.* 1993, Johnson *et al.* 1997). Therefore each individual was equipped with one red ring each to equalize the possible influence of red rings on mating behaviour.

3.2 Analysis of breeding activity

3.2.1 Breeding activity

Overall male breeding activity can be described as the number of new nest-building attempts and overall female breeding activity can be described as the number of nests newly accepted on a given day in the whole colony. Therefore I counted for each nest both the number of new nest-building attempts and the number of nests newly accepted in the whole colony on the day when the nest was started. To obtain an average value for each male, median values for all nests of a male were calculated of both male and female breeding activity at the time of nest-building initiation.

3.2.2 Reproductive success

Male reproductive success was measured as the number of accepted nests, the number of eggs, the number of hatchlings and the number of fledglings. To describe reproductive success in further analysis I used only the number of accepted nests because the number of accepted nests, eggs, hatchlings and fledglings per male correlated well with each other for all three seasons (all $p < 0.0005$). Since it is already known that male reproductive success is mainly determined by the number of nests built (Friedl & Klump 1999, Lawes *et al.* 2002) I computed the residuals of the number of accepted nests against the total number of nests built by a male to mathematically subtract any influence of the number of nests a male built. Thereby I obtained a measure of the component of male reproductive success that was independent of the number of nests built.

3.2.3 Male nest-building activity

The number of nests was defined as the number of completed nests per male for the whole season. Territory tenure was measured in days from the beginning of the first nest until the date of the last nest finished or accepted. This was a very conservative measure and was probably lower than the actual time of territory tenure, but the mere sighting of a male in the colony was regarded as not reliable enough to assign territoriality status to it.

3.2.4 Nest-building speed

Nest-building speed was measured in terms of both building time defined as number of days needed to build one nest, and building delay defined as the time period between the day the last nest of a male was finished and the day the new nest was started. These parameters were recorded for each nest separately. For each male median values were calculated.

3.2.5 Preference for young nests

To obtain a distribution of the expected number of accepted nests per nest age category under the assumption of random female settlement, I calculated for each day the number of available nests (Nav), the number of accepted nests (Nac) and the number of available nests per age category (Nav_{age}). Age categories were defined as age in days. The number of expected accepted nests per day and age category (Nex_{age}) was calculated with

$Nex_{age} = Nac * Nav_{age} / Nav$

The values for Nex_{age} were summarised over the whole season and the obtained distribution of accepted nests per age category under the assumption of random settlement was then compared to the observed distribution of accepted nests per age category using a Kolmogorov-Smirnov-test after Sokal & Rohlf (1995). Additionally, for each nest I recorded the number of days until it became accepted after it was completed.

3.3 Statistics

For statistical tests SPSS software was used. Since variables were not normally distributed, only non-parametric tests were applied. Comparisons of multiple correlation coefficients were calculated after Zar (1999). All tests were two-tailed with $\alpha = 0.05$.

4 Results

4.1 General breeding activity

There were considerable differences between the three breeding seasons (Figure 1). In order to make the three different breeding seasons comparable, the time scale starts in the first week of September in each year. In each of the three study seasons, there was no breeding activity in the first weeks of September, but rainfall occurred during that time. The breeding season in 2001/2002 started and ended earlier than in 2000/2001. In 2002/2003 it started even earlier than in the previous years and showed only one clear breeding peak. In 2000/2001 there was no obvious breeding peak, instead the birds remained at a rather high activity for 4 months, only slightly slowing down in mid January 2001. Only the season 2001/2002 showed a typical course with two strong and distinct breeding peaks. Therefore it needs to be established if there are general patterns in nest-building behaviour that are independent from variations among seasons with respect to the course and intensity of breeding activity. With regard to demand for and supply of nests during a breeding season, Figure 1 illustrates that supply with freshly built nests is most of the time outstripping female demand. The number of previously built, but still available nests must be added to the number of newly built nests on a day to obtain the real number of available nests, which would result in an even larger asymmetry between supply of and demand for nests.

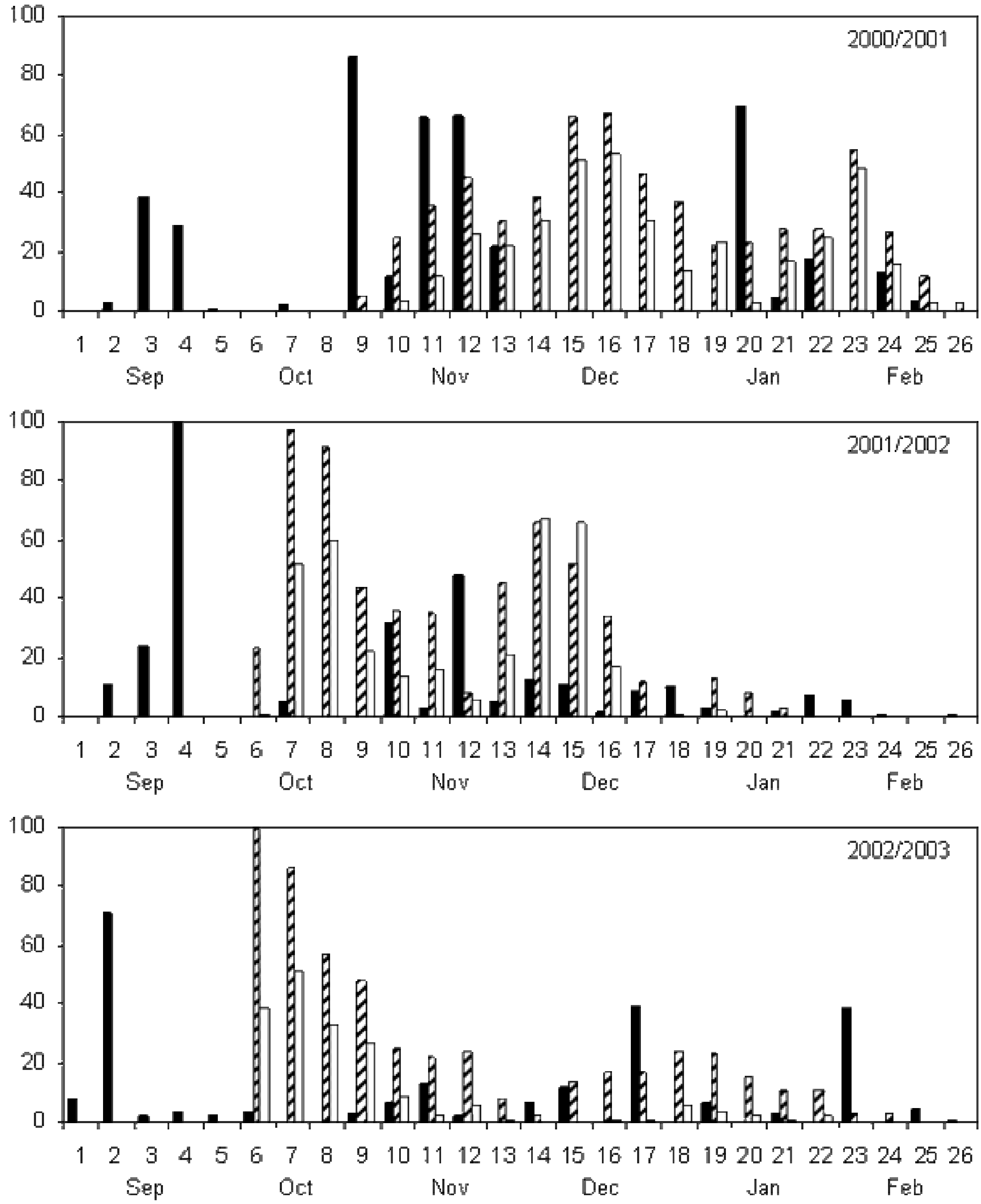


Figure 1 Rainfall in mm (black bars), number of newly built (hatched bars) and newly accepted nests (white bars) per week for the three breeding seasons in the study colony. The numbers on the x-axis indicate weeks after September 1st. The reason for the absence of data on breeding activity in the first weeks is not that they are missing, but because there was no breeding activity.

4.2 Preference for young nests

For each season, the number of expected accepted nests was calculated for each age category and compared to the number of observed accepted nests per age category (Figure 2).

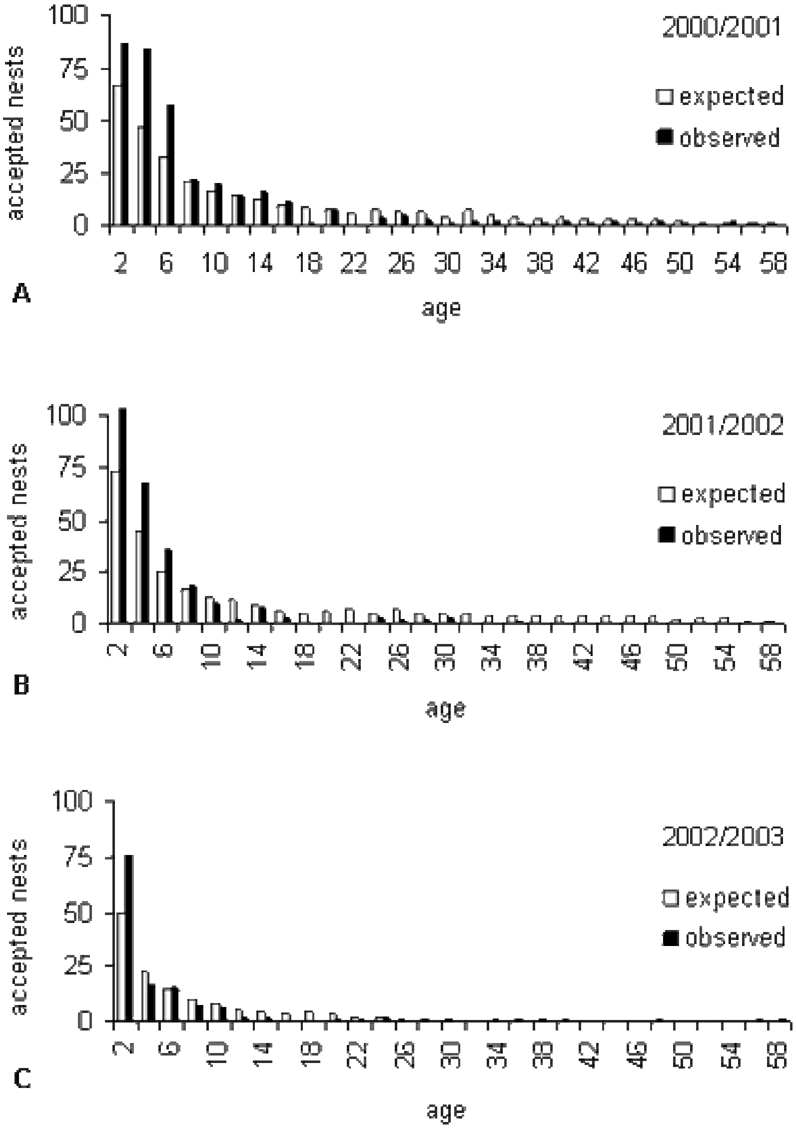


Figure 2 Expected and observed numbers of accepted nests per age category for A) 2000/2001, B) 2001/2002 and C) 2002/2003. In all three seasons more young nests were accepted than expected by chance.

In all three seasons the distributions of the observed number of accepted nests per age category differed from the distribution of the expected number of accepted nests per age category (Kolmogorov-Smirnov: 2000/2001: $d = 0.267 > D_{0.001} = 0.151$, $N = 334$; 2001/2002: $d = 0.306 > D_{0.001} = 0.162$, $N = 288$; 2002/2003: $d = 0.231 >$

$D_{0.001}$ = 0.216, N = 163). As indicated in Figure 2, in the first two seasons nests less than six days old were chosen considerably more often than expected under the assumption of random female settlement, whereas in the last season only two-day-old nests were chosen considerably more often than expected by chance.

4.3 Female nest acceptance and breeding activity

At times of high female breeding activity changes in nest acceptance behaviour might be expected. These changes could affect the average age of accepted nests at different levels of breeding activity. Nests could be accepted that are not yet completely finished, if a female can choose only between such a nest and a nest more than two weeks old. To test this hypothesis, a correlation between acceptance age of nests and female breeding activity was performed. On the other side, females could exert a weaker preference for young nests, if there are not enough young nests available when many females search a nest. This hypothesis was tested with a correlation between breeding activity and variance in the age of accepted nests. To obtain variances in the age of accepted nests for different levels of breeding activity, breeding activity was categorised according to 10 percentiles. All values for breeding activity falling into the first 10% were in the first category, all values for breeding activity falling into the second 10% were in the second category, and so on for all 10% intervals. For each category of breeding activity, variance in the acceptance age of nests was calculated. These variances were tested for a correlation with female breeding activity. Both tests were performed for each season separately with nests as sample units. For each accepted nest, the age at the day of acceptance and breeding activity in terms of the number of nests newly accepted in the whole colony at the day of acceptance were recorded. Since most males had more than one accepted nest, the data on nest age at the day of acceptance were normalized for all nests belonging to the same male and thus for each male separately to account for differences among males. The reason for this normalisation for each male separately is the possibility of differences among males. Some males could get nests accepted only at times of low breeding activity and with a high acceptance age, whereas other males could get nests accepted only at times of high activity and with low acceptance age. This would result in significant correlations that do not reflect a generally valid effect, but instead differences between males. On the other hand, if there are males whose nests have generally low acceptance age, and other males with nests of generally high acceptance age, but for all males separately there is a relation between acceptance age and breeding activity, any generally valid correlation between acceptance age and breeding activity would be blurred and could become undetectable because of too large differences between males. The age of nests at the date of acceptance decreased with increasing breeding activity in all three seasons, whereas variances of the age of nests at the date of acceptance decreased with increasing breeding activity only in the season 2001/2002 (Table 1; Figure 3).

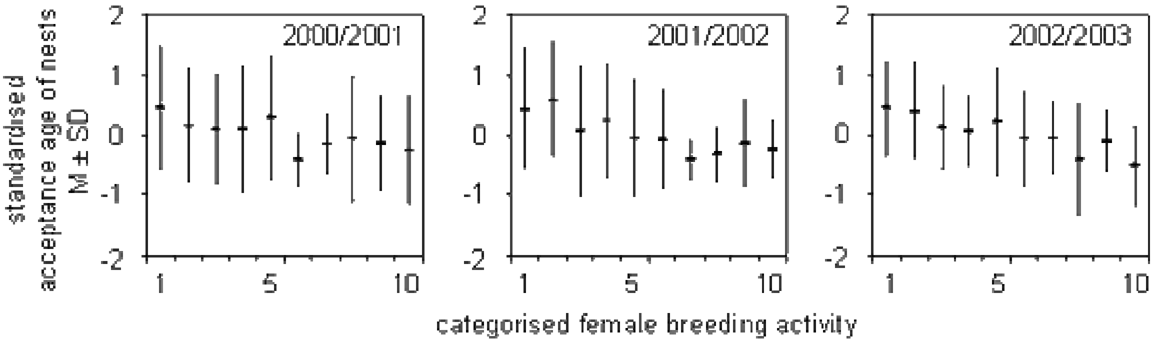


Figure 3 Mean and SD for standardised acceptance age of nests relative to breeding activity for the three study seasons. Female breeding activity was divided into ten categories (percentiles) for each season.

Table 1 Nonparametric correlations between female breeding activity and the age of nests at the date of acceptance. For correlations between breeding activity and variances of the age of nests at the date of acceptance, values of breeding activity were assigned to ten categories according to 10 percentiles.

season		acceptance age of nests	variance of acceptance age of nests
2000/2001	r_s	-0.197	-0.491
	N	334	10
	p	< 0.0005	0.150
2001/2002	r_s	-0.232	-0.806
	N	288	10
	p	0.0005	0.005
2002/2003	r_s	-0.339	-0.248
	N	163	10
	p	< 0.0005	0.489

4.4 Building speed

The variation in nest-building time is illustrated in Figure 4. In all three seasons, most nests were built within 2 days, and only rarely nest-building time exceeded six days.

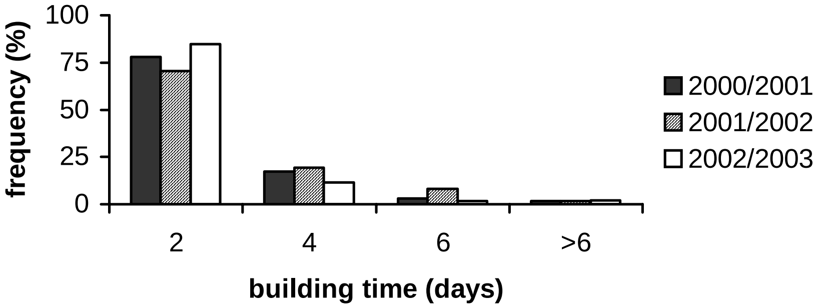


Figure 4 Frequency distribution of building time in days. Most nests were built within two days.

The average nest-building delay over all three study seasons was 5.5 days (see also Figure 5). In the three study seasons the standardised variance (SD / mean * 100) for nest-building delay (2000/2001: 90, 2001/2002: 113, 2002/2003: 135) did not differ consistently from the variance for nest-building time (2000/2001: 146; 2001/2002: 102; 2002/2003: 147).

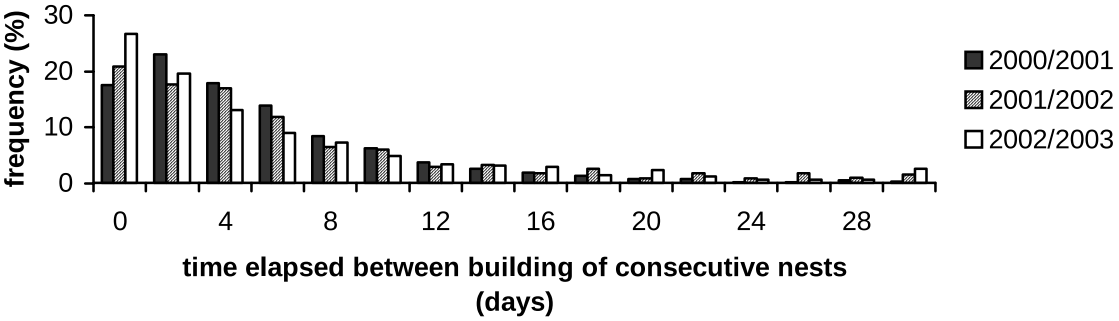


Figure 5 Frequency distribution of the time elapsed from one nest finished until a male started building its next nest.

There were significant differences among males with regard to building delay in two of three seasons, while no significant differences were found with regard to building time (Table 2).

Table 2 Results of Kruskal-Wallis-tests for differences among males for all three study seasons with regard to building time and building delay. Males differed significantly only with regard to building delay.

season		chi-square	df	p
2000/2001	building time	59.59	49	0.143
	building delay	87.34	48	< 0.0005
2001/2002	building time	73.75	63	0.167
	building delay	87.39	63	0.023
2002/2003	building time	52.62	54	0.528
	building delay	64.33	54	0.159

4.5 Timing and synchrony of male and female breeding activity

4.5.1 Nests: female demand and male supply

As shown in Figure 2, female Red Bishops prefer nests less than a week old, and breeding activity varies considerably during a season (Figure 1). These two observations imply that males should build more nests during times of high female breeding activity. Therefore a temporal correlation between variation in male supply of nests and female demand for nests might be expected. To test this assumption, correlation analyses were performed between the number of nests built in a given week in the whole colony and the number of nests accepted two weeks ago, in the previous week, in the same week, in the following week, and two weeks later. There were positive correlations between the number of new nest-building attempts in a given week and the number of accepted nests in all analysed weeks, but none as strong as with the number of accepted nests in the same and the following week (Table 3). There are three possible explanations for this result. First, females might adjust the timing of breeding attempts to the availability of nests, i.e. female nest-acceptance rates are a response to male nest-building rates one week before. Second, males might adjust the timing of nest building to female demand, i.e. male nest-building rates are a response to some cue in female behaviour indicating that females need nests to be able to start their breeding attempts. Third, both male and female breeding activity might be triggered by an external factor such as rainfall. In the following sections these three possibilities are investigated.

Table 3 Spearman-Rho correlations between the number of nests started in the colony in a given week and the number of nests accepted the second previous week, the previous week, the same week, the first week and the second week after that week.

		n nests accepted				
season		-2. week	-1. week	same week	+1. week	+2. week
	r_s	0.525	0.825	0.943	0.849	0.632
2000/2001	p	0.017	< 0.0005	< 0.0005	< 0.0005	0.003
	N	20	21	22	21	20
	r_s	0.143	0.710	0.928	0.820	0.476
2001/2002	p	0.615	0.003	< 0.0005	< 0.0005	0.086
	N	15	15	16	15	14
	r_s	0.294	0.609	0.864	0.876	0.754
2002/2003	p	0.209	0.004	< 0.0005	< 0.0005	< 0.0005
	N	20	20	21	21	20

4.5.2 Male nest-building and preceding rainfall

In the three study seasons, male nest-building activity (number of nests built within a given week) did not significantly correlate with the amount of rainfall one, two, three or four weeks ago (Table 4).

Table 4 Correlations between male nest-building activity and preceding rainfall for the three study seasons.

		rainfall			
season		4 weeks ago	3 weeks ago	2 weeks ago	last 7 days
	r_s	0.183	0.103	0.211	-0.212
2000/2001	p	0.415	0.647	0.345	0.343
	N	22	22	22	22
	r_s	-0.416	-0.368	-0.015	0.143
2001/2002	p	0.109	0.161	0.957	0.597
	N	16	16	16	16
	r_s	0.313	-0.069	-0.024	-0.344
2002/2003	p	0.167	0.766	0.917	0.127
	N	21	21	21	21

To test if rainfall influenced male breeding activity independently from female breeding activity, I calculated a general linear model (GLM) with the number of new nest-building attempts as dependent variable and season as factor. Covariates were rainfall in the previous week, two weeks ago, three weeks ago and four weeks ago, and the number of nests accepted in the next week, because it was the strongest determinant of male nest-building activity. Since there were no consistent correlations between rainfall in previous weeks and male nest-building activity, rainfall for all four previous weeks were included as covariates. Results of the GLM are listed in Table 5. Adjusted R^2 of the resulting model was 0.664. According to the B coefficients, an increase in the number of newly accepted nests in the same week led to more new nest-building attempts.

Table 5 Results of a GLM for the three study seasons with the number of new nest-building attempts per week in the whole colony as dependent variable. Independent variables were the number of accepted nests in the same week and rainfall one, two, three and four weeks ago.

source	B coefficient	sum of squares type III	df	F	partial eta-square	p
corrected model		3003.496	7	17.362	0.704	< 0.0005
intercept	4.535	260.065	1	10.523	0.171	0.002
season	-2.788 / -0.867*	67.021	2	1.356	0.050	0.267
rainfall in the last week	0.002	0.116	1	0.005	< 0.0005	0.946
rainfall two weeks ago	0.010	2.796	1	0.113	0.002	0.738
rainfall three weeks ago	-0.031	10.768	1	0.436	0.008	0.512
rainfall four weeks ago	-0.027	17.934	1	0.726	0.014	0.398
n accepted nests in the same week	1.533	2744.142	1	111.040	0.685	< 0.0005
error		1260.368	51			

* Seasons 2000/2001 and 2001/2002 respectively. B for 2002/2003 is redundant (insufficient degrees of freedom).

4.5.3 Female nest acceptance and preceding rainfall

Because females feed nestlings alone, it is possible that females adjust their breeding activity much more to rainfall than males who try to attract as many females as possible, independent of rainfall. However, correlations between female breeding activity in terms of the number of nests accepted per week and preceding rainfall were not significant for any of the three study seasons (Table 6).

Table 6 Correlation between female breeding activity and rainfall for the three study seasons.

		rainfall			
season		4 weeks ago	3 weeks ago	2 weeks ago	last 7 days
2000/2001	r_s	0.223	0.156	0.219	-0.161
	p	0.318	0.488	0.327	0.473
	N	22	22	22	22
2001/2002	r_s	-0.283	-0.189	0.073	0.226
	p	0.289	0.484	0.787	0.399
	N	16	16	16	16
2002/2003	r_s	0.199	-0.207	-0.109	-0.346
	p	0.387	0.367	0.638	0.124
	N	21	21	21	21

To test if female breeding activity was influenced by preceding rainfall after accounting for male nest-building activity, female breeding activity was regarded as dependent variable in a GLM with season as factor. Covariates were the number of newly built nests and rainfall in the four preceding weeks. Rainfall in all four previous weeks entered the analysis, because no consistent pattern in the relation between preceding rainfall and female breeding activity was observed. Results of the GLM are listed in Table 7. Adjusted R^2 of the resulting model was 0.689. According to the B coefficients, an increase in the number of new nest-building attempts led to more newly accepted nests.

Table 7 Results of a GLM for the three study seasons with the number of newly accepted nests in a given week as dependent variable. Independent variables were the number of new nest-building attempts in the same week and rainfall one, two, three and four weeks ago.

source	B coefficient	sum of squares type III	df	F	partial eta-square	p
corrected model		978.179	7	19.394	0.727	< 0.0005
intercept	-1.427	6.054	1	0.840	0.016	0.364
season	1.487/1.141*	21.389	2	1.484	0.055	0.236
rainfall in the last week	-0.003	0.277	1	0.038	0.001	0.845
rainfall two weeks ago	-0.004	0.446	1	0.062	0.001	0.804
rainfall three weeks ago	0.023	6.028	1	0.837	0.016	0.365
rainfall four weeks ago	0.029	22.921	1	3.181	0.059	0.080
number of new nest-building attempts	0.447	800.088	1	111.040	0.685	< 0.0005
error		367.476	51			

* Seasons 2000/2001 and 2001/2002 respectively. B for 2002/2003 is redundant (insufficient degrees of freedom).

4.6 Adjustment of building speed

Considering that females prefer young nests and that a breeding course typically shows two distinct peaks of breeding activity it can be assumed that males increased their building speed with higher breeding activity. Building speed can be increased either by building nests faster or by quickly building one nest after another. Building time and building delay for each nest were normalized for each male separately to eliminate possible masking effects of inherent differences in building speed among males on the general pattern investigated, i.e. whether nest-building speed is adjusted to female demand for new nests, analogous to the analysis of a correlation between acceptance age of nests and female breeding activity above. Building time and building delay of all nests were negatively correlated with the number of accepted nests in 2001/2002 and 2002/2003, but not in 2000/2001. Effect size was generally small, with the exception of building delay in 2002/2003 (Table 8).

Table 8 The number of accepted nests in the colony in the week a nest was newly started in correlation to building time of that nest and time elapsed since the last nest of that male was finished (building delay).

Season		building time	building delay
2000/2001	r_s	-0.073	-0.002
	p	0.091	0.962
	N	530	465
2001/2002	r_s	-0.219	-0.334
	p	< 0.0005	< 0.0005
	N	501	457
2002/2003	r_s	-0.188	-0.628
	p	< 0.0005	< 0.0005
	N	449	412

These results raised the question if males that can better adjust their building delay to actual breeding activity profited from higher reproductive success.

4.6.1 Male nest-building adjustment and reproductive success

Since building time and building delay were shorter during high breeding activity, males with a better adjustment of their building speed to female breeding activity could profit from higher reproductive success. To quantify adjustment of building delay to female breeding activity for each male I calculated the relation of nests built during high breeding activity to the total number of nests built by each male. Males with a shorter building delay during high breeding activity than during low breeding activity should have a higher ratio of nests built during high breeding activity to their total number of nests built. High breeding activity was defined as a high number of newly accepted nests in the week a nest was built. As cut-off value to define high breeding activity I used the upper quartile of the number of newly accepted nests. Since the influence of the number of nests built by a male on the number of accepted nests is well demonstrated (Friedl & Klump 1999, Lawes *et al.* 2002), the residuals of the number of accepted nests against the total number of nests built by a male were used to describe male reproductive success (see Methods). Only in the season 2001/2002, males with better building speed adjustment had higher reproductive success. In the two other seasons, such a positive effect was not significant (Table 9). As additional measurements of male nest-building adjustment I calculated for each male correlation coefficients of both building time and building delay with female breeding activity. These correlation coefficients indicated how accurately males were adjusting their building speed (building time and building delay) to female breeding activity. Correlation coefficients did not differ among males in any of the three study seasons (comparisons of multiple correlation coefficients after Zar (1999); all $p > 0.3$). These correlation coefficients for each male (i.e. the accuracy with which a male adjusted his building speed to female breeding activity) were tested for correlations with reproductive success. In the season 2002/2003, males with a less accurate adjustment of building time had higher reproductive success (Table 9).

Table 9 Nonparametric correlations of three different measures of male adjustment to female breeding activity with male reproductive success. The three measures taken for each male were (i) the ratio of nests built during high activity to all nests built (adjustment), (ii) correlation coefficients of building time with female breeding activity for each male, and (iii) correlation coefficients of building delay with female breeding activity for each male.

Season		adjustment	building time correlation coefficient	building delay correlation coefficient
2000/2001	r_s	0.211	0.056	-0.105
	N	42	48	46
	p	0.179	0.707	0.486
2001/2002	r_s	0.305	0.134	-0.032
	N	54	62	58
	p	0.025	0.300	0.809
2002/2003	r_s	0.259	-0.290	0.066
	N	52	53	52
	p	0.064	0.035	0.642

5 Discussion

5.1 Dynamics of the Red Bishop's mating market

The biological market described here dealt with changes in demand for and supply of nests during a breeding season. Several characteristics could be identified governing the exchange value of nests. Both male supply of and female demand for nests vary over time within a breeding season. Peaks in male and female breeding activity can occur within only few days, as at the onset of the breeding season in 2001/2002 and 2002/2003. In all three seasons, male nest-building activity as well as female breeding activity could increase rapidly at any time during the breeding season. Male nest-building activity and female breeding activity were closely linked, although not perfectly simultaneous. The strongest correlation with the number of new nest-building attempts in the colony in a given week was found for the number of accepted nests in the same week, but the number of accepted nests in the following week and, somewhat weaker, in the previous week also correlated with male nest-building activity.

Males could estimate female demand for new nests by the number of females visiting the colony in search for a nest and use this as a cue to adjust nest-building activity. It is much easier for a male to estimate the number of females visiting the colony than the number of newly accepted nests in the whole colony, because nests in other territories can not easily be inspected by a male without being chased away immediately by the territory owner. Visiting females looking for a suitable nest for a new breeding attempt show a typical behaviour that elicits a typical response by males. As soon as a visiting female perches somewhere in a territory, the territory owner as well as its neighbours immediately engage in courtship behaviour (Emlen 1957). Most of the time, the female will approach one male and inspect the nest to which the male is leading the female. The number of females visiting a colony, perching in territories and inspecting nests advertised by males could be a good indicator of female breeding activity in the next days and might therefore be used by males to adjust their nest-building effort. Even with the observed considerable changes in male and female breeding activity, male supply of nests was at any time outstripping female demand for nests. This result illustrates the role of females as the choosing traders and the role of males as the chosen traders in this mating market. It also indicates potential for sexual selection (Parker *et al.* 1972, Clutton-Brock & Vincent 1991), given that males try to attract as many females as possible whereas females of this highly polygynous species (Friedl & Klump 1999) can choose based on a direct comparison of males and nests in a colony.

Further on, nest-building as a condition-dependent behaviour can be adjusted to the current market situation. In contrast, morphological traits can not quickly be adapted to changing market conditions (Noë & Hammerstein 1994). Building time and building delay became shorter with increasing female demand in two of three seasons. There are two possible explanations for these changes in building time and building delay. Either males generally differ in their ability to build fast, and at high breeding activity there are more males building fast than at low breeding activity, or males adjust their building time and building delay to current female demand. In other words, either there are differences between the traders of the chosen class or the chosen class might be able to vary production costs of their trading commodity according to demand. The data presented here suggest a combination of both explanations. As shown, males differed with respect to building delay, but not building time. On the other side, because differences between males were eliminated by means of data normalization, the observed adjustments of building time and building delay to the intensity of female breeding activity were generally valid.

It might be expected that males that better adjust nest-building to female breeding activity obtain increased reproductive success. However, the results are equivocal. In only one season, males with a higher ratio of nests built during high breeding activity to nests built during low breeding activity profited from higher reproductive success. In another season, males with a less accurate adjustment of building time profited from higher reproductive success. A possible explanation for this result is given by the observation that generally faster building males have higher reproductive success (see Chapter 1). However, males did not differ in their building time. Some males might always build fast, whereas other males build slower at times of low breeding activity and faster at times of high breeding activity. Still, the males that always build fast would have higher reproductive success than males that built fast only at times of high breeding activity.

5.2 Nests as a commodity in a biological market

Red Bishop nests are well suitable to be regarded as a commodity in a market, because there is no other function of nests reported in Red Bishops than protection of eggs and nestlings (Craig 1974, Friedl & Klump 1999, 2000, Lawes *et al.* 2002). Additionally, nests are solely traded for mating opportunities. Nests are a commodity of members of the chosen class and chosen by females according to the age of a nest. Being chosen is a feature necessary for nests to qualify as a commodity in a market model. Cheating is not possible, because a nest can not be ‘faked’ or withdrawn from the market after it is offered. In comparison, grooming in primates is not strictly defined and is an agglomeration of several functionally different behaviours with context-dependent differences in its value and its pay-off as a trading commodity (Colmenares *et al.* 2002). Further on, grooming in baboons can be traded for a variety of other goods (Barrett *et al.* 1999), rendering a biological market approach more complicated.

According to Noë & Hammerstein (1994, 1995), cheating is not an essential feature of biological market models. This applies better to the chosen trader than to the choosing trader, if the chosen trader offers its commodity first and the choosing trader offers its exchange commodity only later. Once the commodity offered by the chosen trader is accepted and given away, the choosing trader can cheat and not give its commodity in exchange as expected by the chosen trader. In Red Bishops for example, females accept a nest of a male and engage in extra pair copulations resulting in 30.5% of all broods containing extra pair young (Friedl & Klump 1999). Cheating can especially occur in breeders and helpers, if the benefit for a helper is a potential future mating opportunity with a breeder (Reyer 1986). In this case the commodity offered by the helper can be regarded as a bet on receiving a commodity by the breeder in the future. The helper merely increases his chances to receive this commodity, but the transaction is not for granted.

5.3 Triggers of breeding activity

In contrast to many other bird species, a typical breeding season in the study colony of Red Bishops lasts for four to five months with usually two, sometimes only one breeding peak (Friedl 2002). The onset of a breeding season, indicated by males moulting into the typical breeding plumage, subsequent nest-building activity and egg-laying depends on external factors (e.g. rainfall) and internal factors (e.g.

hormone levels). According to other studies (Brooke 1966, Craig 1982, Lloyd 1999, Friedl 2002), female breeding activity is positively connected to preceding rainfall. In this study, though, there was no relation between female breeding activity and rainfall. This is at first sight surprising and different from the findings by Friedl (2002), but females can only accept nests after males built nests, and then evaluate preceding rainfall patterns. The importance of rainfall for breeding activity is probably more prominent if analysed on a larger time scale than done here, months or even seasons, as in Brooke (1966), Craig (1982), and Lloyd (1999). Weeks were apparently a too short time span to detect the influence of rainfall on breeding activity in the three study seasons for the investigated colony. The importance of overall rainfall during a breeding season is well demonstrated (Brooke 1966, Craig 1982, Lloyd 1999, Friedl 2002) and was not an aim of this study. The model presented here aimed at illustrating the strong links between male nest-building activity and female breeding activity, and it was demonstrated that male and female breeding activity on a weekly scale are connected even after taking preceding rainfall into account.

5.4 Preference for young nests

A crucial demand on male nest-building behaviour is the decrease in acceptance probability of a nest with increasing nest age. Hansen (2001) showed that newly accepted nests were younger than the median age of all available nests, summarized over all days within a season on which nests became accepted. Hansen (2001) argued that the day of first egg discovery was a very conservative measure for the date of nest acceptance and therefore estimated the age of acceptance not only with this conservative measure, but also with a less conservative measure, where the date of nest choice was assumed to be two days prior to the date of first egg acceptance. There is no other clue than the date of first egg appearance to determine the date of nest acceptance. Therefore I used the date of first egg appearance as the date of nest acceptance. Nests not older than a week were more often accepted than expected by chance alone. This implies that it is not sufficient for a male to have not yet accepted nests available, but nests that are less than a week old. A market model in which supply of a commodity is larger than demand for this commodity postulates that this commodity can be chosen according to certain properties, and a member of the class offering this commodity can not force a member of the other trading class into a deal. With the higher acceptance probability of younger nests, this condition is fulfilled by the nests of male Red Bishops. A possible functional explanation for this preference for young nests is durability of nests as proposed by Collias & Victoria (1978), who found a preference of Village Weavers for fresh, green nests. Painting an old, brown nest green did not raise its probability of becoming accepted. A nest has to last for about a month until the nestlings fledged, and a young nest might have a higher probability of lasting for the following month. Another reason might be that young nests are easier to manipulate and work with than older nests, because fresh green fibres are more flexible, considering that females line out the inside of nests and incorporate more fibres (Skead 1956). In this study on Red Bishops as well as in the study by Collias & Victoria (1978) on Village Weavers it could not be distinguished if nests were accepted by females independently of male advertisement, but this does not change consequences for male building behaviour. It does not matter why younger nests have a higher probability of becoming accepted, because for demands on nest-building behaviour the fact itself is important, not the cause of it. It is possible that males preferably advertise young nests because of a female preference for young nests. If a male is advertising a nest to a visiting female, the female has a choice of nests because it can reject this nest and inspect another male with its advertised nest. This ability to switch trading partners is a crucial feature of working biological markets.

The already high probability for younger nests to become accepted increased further with female breeding activity, as indicated by a decrease in the average acceptance age of nests with increasing breeding activity. This result might be due to the fact that more young nests are available at times of high breeding activity because males build more nests. The fact that in one season variance of the acceptance age of nests decreased with increasing female breeding activity supports this explanation. Either some or all males seem to increase their supply of young fresh nests and thus adjust their nest-building effort and speed to female breeding activity.

5.5 Conclusions

In conclusion, the breeding system of Red Bishops was found to be suitable for the application of a theoretical framework based on biological market models, because the contribution to offspring survival differs distinctly for males and females.

Males only provide nests, females provide incubation of eggs and feeding of nestlings. On the mating market of Red Bishops, nests are traded against mating opportunities, incubation of eggs and feeding of nestlings. The commodities offered by males and females are asymmetrical with regard to demand and supply. Supply of nests is always larger than demand for nests, implicating that supply of incubation of eggs and feeding of nestlings is always smaller than demand for it. Males as a trading class are chosen according to the age of their nests offered. With regard to nests, supply and demand change over time within a breeding season, and production costs for nests increased with demand for nests as indicated by shortened building times and building delays.

6 References

Axelrod, R., Hamilton, W.D. 1981. The evolution of cooperation. Science 211: 1390-1396

Barrett, L., Henzi, S.P., Weingrill, T., Lycett, J. E., Hill, R. A. 1999. Market forces predict grooming reciprocity in female baboons. Proceedings of the Royal Society London B 266(1420): 665-670

Brooke, R.K. 1966. Nuptial moult, breeding season and clutch size of Rhodesian Red Bishops *Euplectes orix* and congeners in relation to rainfall. Ostrich sup 6: 223-235

Bshary, R. 2001. The cleaner fish market. In: Economics in Nature, eds Noë, R., van Hooff, J.A.R.A.M., Hammerstein, P. Cambridge University Press, Cambridge, pp. 146-172

Bshary, R., Grutter, A.S. 2002. Experimental evidence that partner choice is a driving force in the payoff distribution among cooperators or mutualists: the cleaner fish case. Ecology Letters 5(1): 130-136

Clutton-Brock, T.H., Vincent, A.C.J. 1991. Sexual selection and the potential reproductive rates of males and females. Nature 340: 463-465

Collias, N.E., Collias, E.C. 1978. Cooperative breeding behavior in the white-browed sparrow weaver. Auk 95: 472-484

Collias, N.E., Collias, E.C. 1984. Nest Building and bird behavior. Princeton University Press, Princeton, N.J.

Collias, N.E., Victoria, J.K. 1978. Nest and mate selection in the village weaverbird (*Ploceus cucullatus*). Animal Behaviour 26: 470-479

Colmenares, F., Zaragoza, F., Hernandez-Lloreda, M.V. 2002. Grooming and coercion in one-male units of hamadryas baboons: Market forces or relationship constraints? Behaviour 139(11-12): 1525-1553

Craig, A.J.F.K. 1975. Hodenreife, Prachtkleid und Schnabelfärbung beim Oryxweber (Euplectes orix). Journal of Ornithology 116: 87-93

Craig, A.J.F.K. 1982. The breeding season of the Red Bishop. Ostrich 53: 112-114

Craig, A.J.F.K 1985. Breeding condition of male red bishops under artificial photoperiods. Ostrich 56: 74-78

Dunbar, R.I.M. 2001. The economics of male mating strategies. In: Economics in Nature, eds Noë, R., van Hooff, J.A.R.A.M., Hammerstein, P. Cambridge University Press, Cambridge, pp. 245-269

Eckardt, W., Zuberbühler, K. 2004. Cooperation and competition in two forest monkeys. Behavioral Ecology 15(3): 400-411

Emlen, J.T.Jr. 1957. Display and mate selection in the whydahs and bishop birds. Ostrich 28: 202-214

Friedl, T.W.P. 2002. The effect of rainfall on the breeding behaviour of the Red Bishop, *Euplectes orix*. Ostrich 73(3-4): 181-184

Friedl, T.W.P., Klump, G.M. 1999. Determinants of male mating success in the red bishop (*Euplectes orix*). Behavioural Ecology and Sociobiology 46: 387-399

Friedl, T.W.P., Klump, G.M. 2000. Nest and mate choice in the red bishop (*Euplectes orix*): female settlement rules. Behavioral Ecology 11: 378-386

Hansen, L.H. 2001. Mate choice and vocalisation in the red bishop (*Euplectes orix*). MSc Thesis, University of Copenhagen

Henzi, S.P., Barrett, L. 2002. Infants as a commodity in a baboon market. Animal Behaviour 63(5): 915-921

Lawes, M., Slotow, R., Andersson, S. 2002. Male nest building but not display behaviour directly influences mating success in the polygynous Red Bishop, *Euplectes orix*. Ostrich 73(3-4): 87-91

Lloyd, P. 1999. Rainfall as a breeding stimulus and clutch size determinant in South African arid-zone birds. Ibis 141: 637-643

Lovegrove, B. 1993. The living deserts of southern Africa. Fernwood Press, Vlaeberg

Maclean, G. L. Ed. 1993 Roberts' Birds of Southern Africa, 6th Edition, John Voelcker Bird Book Fund, Cape Town, South Africa

Noë, R. 2001. Biological markets: partner choice as the driving force behind the evolution of mutualisms. In: Economics in Nature, eds Noë, R., van Hooff, J.A.R.A.M., Hammerstein, P. Cambridge University Press, Cambridge, pp. 93-118

Noë, R., Hammerstein, P. 1994. Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behavioural Ecology and Sociobiology 35: 1-11

Noë, R., Hammerstein, P. 1995. Biological Markets. TREE 10(8): 336-339

Nowicki, S., Peters, S., Podos, J. 1998. Song learning, early nutrition and sexual selection in songbirds. American Zoologist 38: 179-190

Ostrom, E. 2001. Social dilemmas and human behaviour. In: Economics in Nature, eds Noë, R., van Hooff, J.A.R.A.M., Hammerstein, P. Cambridge University Press, Cambridge, pp. 23-41

Parker, G.A., Baker, R.R., Smith, V.G.F. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. Journal of Theoretical Biology 36: 529-553

Pellmyr, O., Huth, C.J. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. Nature 372(6503): 257-260

Reyer, H.U. 1986. Breeder-helper-interactions in the pied kingfisher reflect the costs and benefits of cooperative breeding. Behaviour 96(3-4): 277-303

Savalli, U.M. 1993. The timing of breeding and moult of the Yellowmantled Widowbird *Euplectes macrourus* in western Kenya. Ostrich 64: 49-56

Seyfarth, R.M. 1977. A model of social grooming among adult female monkeys. Journal of Theoretical Biology 65: 671-698

Skead, C.J. 1956. A study of the Red Bishop bird. Ostrich 27: 112-126

Stopka, P., Macdonald, D.W. 1999. The market effect in the wood mouse, *Apodemus sylvaticus*: Selling information on reproductive status. Ethology 105 (11): 969-982

Zar, J.H. 1999. Biostatistical analysis. 4th edition. Prentice Hall, New Jersey

Zur Homepage der Dissertation

Chapter 4

An automated analysis of avian forebrain activity in relation to nest-building in Red Bishops (*Euplectes orix*)

Abstract

Nest-building in weaverbirds is a behaviour comprising complex sequential motor patterns. I identified forebrain regions involved in the sensory and motor control of nest-building in yearling male Red Bishops (*Euplectes orix*) by analysing changes in forebrain activity measured by ZENK protein expression after nest-building behaviour. Birds were allowed to manipulate nest-building material for 30 min. Brain activity was measured as the number of cell nuclei showing expression of the Immediate Early Gene product ZENK in 300 x 300 µm sampling frames covering the whole forebrain in eight analysed parasagittal sections per hemisphere. For each section, the number of stained cell nuclei was automatically counted for all frames. Then the forebrain was divided into 128 regions of interest per hemisphere and all sampling frames falling into the same region were used to automatically calculate mean ZENK protein expression in that region. ZENK protein expression increased after nest-building in the frontal hyperpallium apicale from 0.5 mm to 2.0 mm from median in the right hemisphere. In the caudal mesopallium a decrease in ZENK expression with nest-building was observed at 2.0 mm from median in the left hemisphere. In the nidopallium, three different regions showed changes in ZENK expression after nest-building. The first region was located in the left hemisphere frontal and from 3.0 mm to 4.0 mm from median and showed an increase in ZENK expression. The second region was located in the left hemisphere intermediate in the frontocaudal axis and 1.5 mm to 2.5 mm from median, with a decrease in ZENK after nest-building. The third region in the nidopallium was in the right hemisphere caudal and 3.0 to 4.0 mm from median in the right hemisphere with an increase in ZENK after nest-building. In the Medial Striatum, a region in the left hemisphere located caudal and 0.5 to 1.5 mm from median showed a decrease in ZENK expression after nest-building. All above regions with a significant change in ZENK expression were tested for differences between experimental and control animals in their differences between hemispheres. There was a significant difference between hemispheres only in the frontal hyperpallium. Building birds had a larger difference in ZENK expression between hemispheres than control birds in this region, caused by higher levels of ZENK expression in building birds in the right hemisphere.

1 Introduction

The function of numerous avian forebrain regions has been well studied in the contexts of song (reviews in Brainhard & Doupe 2002, Mello 2002), food storage (e.g. Healy & Krebs 1993, Smulders & DeVoogd 2000), filial imprinting (e.g. McCabe & Horn 1994, Bolhuis, Cook & Horn 2000), sexual imprinting (Bischof 2003, Sadananda & Bischof 2004) and processing of sexual stimuli such as copulation and social proximity (Ball *et al.* 1997, Ball & Balthazart 2001). As a general principle, Jarvis & Mello (2000) postulated that for any complex behavioural pattern, nuclei within each of the major avian forebrain subdivisions - hyperpallium, mesopallium, nidopallium, arcopallium, medial and lateral striatum - are participating in the processing of sensory input and control of behavioural output. A typical behaviour in birds requiring high levels of sensory and motor control is nest building (Collias NE & Collias EC 1984, Hansell 2000). It is not known which forebrain areas are involved in learning, sensory control and execution of this complex behavioural pattern. By looking at neural mechanisms underlying nest-building behaviour, new insights into the functional role of regions of the avian forebrain can be gained. This study wants to provide a first indication as to which regions of the forebrain are involved in nest-building behaviour in Red Bishops (*Euplectes orix*), a weaverbird widespread in sub-Saharan Africa.

1.1 Nest building in weaverbirds

Weaverbird nests are typically constructed by weaving together small twigs or leaves or stripes of leaves and are suspended in reeds, grass stems or trees (MacLean 1993). Some of the techniques used by weaverbirds to knot and weave these fibres together are highly sophisticated and resemble human knotting and weaving (Collias NE & Collias EC 1984). Nest frames are built by males only (Skead 1956, Emlen 1957, Collias EC & Collias NE 1964). Nest-building in weaverbirds needs to be learned in the first two years of life (Collias EC & Collias NE 1964, Collias EC & Collias NE 1973, Collias NE & Collias EC 1984). Experiments with young male Village Weavers (*Ploceus cucullatus*) demonstrated that at the age of only a few months, every material that can be manipulated with a beak is used for behavioural patterns resembling nest-building such as moving it back and forth in the beak with vibrations or trying to weave it into the mesh-wire of aviaries. Later on in their development they reject artificial materials if natural materials (fibres and strips of leaves) are available (Collias EC & Collias NE 1964). Young male Village Weavers at the age of one year reared without access to nest-building material did not weave fibres into vegetation or mesh wire after they were supplied with nest-building material. These deprived males managed to weave their first stitches only after three weeks of access to nest-building material (Collias EC & Collias NE 1964). An impaired ability to manipulate strips was also observed in young males that were deprived of normal nest-building material from the age of seven weeks who wove fewer strips than control males (Collias EC & Collias NE 1973). Collias EC & Collias NE (1964) state that young weaverbirds learn nest-building through experience and exercising and that tuition is not required for the development of species-typical nest-building behaviour. On the question of what exactly young male weavers learn, Collias EC & Collias NE (1964) suggested that they have to learn particular motor skills like ripping off stripes from grass, reed or bulrush leaves, the actual weaving and knotting, and effective sequences of these motor patterns. In this study, forebrain activity in relation to nest-building was analysed in yearling male Red Bishops, a species that shows the outlined characteristics in weaverbird nest-building behaviour.

1.2 IEGs as a tool to measure brain activity

The investigation of brain area activity by the expression of Immediate Early Genes (IEGs) is an established technique (Chaudhuri 1997) and has been applied in a variety of contexts (Long & Salbaum 1998, Clayton 2000), particularly song learning and production in birds (review in Mello 2002). A number of genes show rapid response to stimulation and are classified as IEGs. One of these genes is *zenk*. *Zenk* (Mello *et al.* 1992) is an acronym for zif-268 (Christy *et al.* 1988), egr-1 (Sukhatme *et al.* 1988), ngfi-a (Milbrandt 1987) and krox-24 (Lemaire *et al.* 1988) and was first described in mice and humans. In the following, *zenk* refers to the gene and ZENK to the protein. ZENK is a transcription factor modulating the expression of other genes, resulting in modulations of synaptic connections and is thus involved in long-term memory formation and neural plasticity (Chaudhuri 1997, Clayton 2000). The ZENK protein is evolutionary conserved, thus very similar across taxa (Long & Salbaum 1998) and commonly used to map brain activity.

The aim of this study was to quantitatively analyse ZENK expression in a large number of forebrain regions for both hemispheres after nest-building. New information about functional bird brain organisation shall be provided by investigating brain activity in relation to a behavioural pattern that needs to be learned and requires complex sensory and motor control. For several sections the whole forebrain was analysed, because IEG expression regulation by nest-building activity in birds has not been investigated before and a large-scale screening of the forebrain was regarded necessary. In previous studies on IEG expression, the number of stained cell nuclei was usually counted in only one or two sampling frames covering a region of interest, and the number of investigated regions was seldom larger than ten. For this study, a new method was developed by which contiguous sampling frames were placed on a whole section of a forebrain and brain activity in defined regions of interest was automatically determined by analysing all sampling frames falling into a region of interest. Throughout this article, brain areas are labelled as agreed on by the Avian Brain Nomenclature Forum held at Duke University in July 2002 and published in Reiner *et al.* (2004). The rationale for the new nomenclature is also explained in Jarvis *et al.* (2005).

1.3 Abbreviations

A	arcopallium
CMM	caudal medial mesopallium
HA	hyperpallium apicale
Hp	hippocampus
HVC	higher vocal centre, HVC used as proper name
IMM	intermediate medial mesopallium
L	field L of the nidopallium
LMAN	lateral magnocellular nucleus of the anterior nidopallium
LSt	lateral striatum
M	mesopallium
MMAN	medial magnocellular nucleus of the anterior nidopallium
MSt	medial striatum
N	nidopallium
NC	nidopallium caudale
NCM	caudal medial nidopallium
RA	nucleus robustus of the arcopallium

2 Methods

2.1 Experimental design

Experiments were conducted with nine yearling male Red Bishops. These young males were separated from their mothers as soon as they could feed themselves (about 4 weeks after fledging) and were then reared without access to nest-building material, visually separated from adult males. Pilot experiments showed that the brain is fully developed at the age of about one year, but not at the age of 2 to 4 months. Therefore and because motivation to manipulate nest-building material should be highest during a breeding season, the young males were tested at the age of about one year during their first breeding season. The aim was to test young males at the beginning of their learning phase that usually commences as soon as a young weaverbird is able to weave fibres, which is at about seven weeks of age in Village Weavers (Collias EC & Collias NE 1973). Therefore the beginning of the learning phase had to be experimentally delayed. As was observed by Collias EC & Collias NE (1964) for yearling male Village Weavers deprived of nest-building material, yearling male Red Bishops were unable to manipulate nest-building material in the first week after nest-building material was supplied. Thus, it was safe to assume that learning commenced for these deprived birds only in their first breeding season and not in their first months of life. In order to obtain levels of ZENK expression related to nest-building activity that are sufficiently high for an analysis, the young males had to manipulate nest-building material frequently. Therefore, the young males were exposed to nest-building material until they manipulated fibres and wove them with stitches on twigs or into the mesh wire of the aviary. Four weeks after first exposure to nest-building material, all of the young males were able to manipulate fibres. None of the nine males managed to build a complete nest, but all of them wove fibres on twigs or into the mesh wire of the aviary. For the experiments, young males were placed in an aviary without access to building material for 24 hours to get accustomed to the new environment and to let subside nest-building related ZENK expression. The actual experiments took place between 5am and 8am, just after sunrise. Nest-building material was supplied and behaviour monitored. Recorded behavioural patterns were the number of manipulations, time spent with manipulations, and time spent singing. If the experimental animal manipulated more than 10 fibres within 30 min, the experiment was valid, otherwise it was cancelled. 90 min after onset of building activity the animal was perfused to obtain peak ZENK protein expression. *Zenk* mRNA levels peak 15-30 min after stimulus presentation, ZENK protein levels consequently peak later, 1-3 hours after stimulus presentation (Mello & Clayton 1994, Mello & Ribeiro 1998, Bolhuis, Zijlstra *et al.* 2000). Control animals were treated like experimental animals, the difference being that the nest-building material supplied could be seen, but not reached. In one animal, perfusion and subsequent staining failed. This animal was excluded from further analysis, resulting in 5 experimental animals and 3 control animals. One experimental animal, 148 TUM, was not properly perfused and only few forebrain areas could successfully be stained for ZENK expression. Of this individual, only areas with successful ZENK staining were analysed. Table 1 gives an overview of the 8 investigated individuals and their activity patterns.

Table 1 Overview of the activity patterns of experimental and control animals.

ring nr	experimental / control	building (sec)	singing (min)	total observation time (min)
136 TUM	experimental	330	2	120
140 TUM	experimental	1590	0	120
144 TUM	control	60	3	110
145 TUM	experimental	540	8	130
148 TUM	experimental	790	0	150
149 TUM	control	20	0	110
152 TUM	experimental	440	14	120
153 TUM	control	90	0	130

2.2 Immunocytochemistry

The staining protocol followed established protocols (e.g. Mello & Ribeiro 1998, Bolhuis, Zijlstra *et al.* 2000, Serries 2000). First, a polyclonal rabbit primary antibody against ZENK (Santa Cruz C-19) was added, second, a secondary biotinylated goat anti-rabbit antibody was binding on the rabbit antibody, and third, an avidin-biotin complex with one biotin carrying a horseraddish peroxidase (ABC) was added. This peroxidase was detected by staining with diaminobenzidine (DAB), intensified by nickel sulfate hexahydrate. This staining procedure resulted in stained nuclei of dark brown colour and a light-grey background. For the secondary biotinylated antibody and the ABC-solution, Elite ABC kits with a goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA) were used. To block any existing avidin and biotin, avidin and biotin blocking solutions (Vector Laboratories) were used. Birds were first anaesthetised with an overdose of Narcoren, 30 µl of a 16% dilution. They were perfused with 50ml physiological saline solution containing 0.5% $NaNO_2$ to prevent blood coagulation and to replace blood, then with 250 ml Zambonis Fixans consisting of 4% paraformaldehyde in 0.1M phosphate buffer (PB) with 15% saturated Picrinic acid. After perfusion, brains were submerged in Zambonis Fixans with 30% sucrose for 12 hours. After embedding in TissueTek (Sakura Finetek, Torrance, CA) and freezing, 50 µm serial parasagittal sections were cut on a cryostat. Sections were washed overnight in 0.1M PB. The next day, sections were washed for 20 min in 0.1 M PB with 1% H_2O_2, washed for 2x15 min in 0.1M PB with 0.1% Triton X-100, and incubated for 60 min with avidin blocking solution and 1.5% normal goat serum. Subsequently, sections were incubated for 36 hours in primary antibody diluted 1:10,000. Sections were then incubated for 70 min with secondary antibody (1:300 diluted stock solution) and normal goat serum (1:150 diluted stock solution) and incubated for 30 min in ABC-solution (solution A and solution B diluted 1:100). All of the above solutions were made in 0.1M PB with 0.1% Triton X-100. From the incubation with avidin blocking solution and normal goat serum onwards, each step was followed by washing for 2x15 min in 0.1M PB. For developing, sections were incubated in 0.03% DAB, 1 mM $NiSO_4$ and 0.03% H_2O_2 in 0.1 M Tris-buffer for two to three minutes. Sections were then allowed to dry, mounted on slides and covered with Depex (Fluka). In some staining procedures of other studies, sections are mounted first and then stained, but in this procedure, sections were mounted on slides only in the last step, and not right after cutting sections on the cryostat. This eliminated the possibility of partial staining due to section location on the slide combined with a slightly tilted workbench leading to sections being unequally covered in antibody.

2.3 Image analysis

This step was carried out semiautomatically with AnalySIS software, a Zeiss Axioskop 2 motplus equipped with a computer controlled Maerzhäusser stage using a 10x objective (ZEISS Plan-Neofluar 10x/0.30 / 44 03 30) and a DSP colour CCD camera (JAI CV-S3200). Per animal and hemisphere, eight sections were analysed.

Due to only partial tissue fixation in one animal, most regions could only be analysed for seven animals. The distance between stained sections was 0.5 mm. The first section was located at 0.5 mm from median and the last section was located at 4.0 mm from median for each hemisphere. First, of each section a picture for overview was taken with the camera mounted on the microscope. For the overview, a 1.25x objective was used giving a resolution of 10.19930 µm/pixel. Based on this picture, frame positions were defined, so that the forebrain was completely covered. Frames measured 300 x 300 µm and were adjacent to each other. With the overview picture, frame positions could also be matched to regions of interest for each section (see Figure 1). The number of stained cell nuclei per 300 x 300 µm frame was counted with the frames covering the whole forebrain (see Figure 1) using a 10x objective giving a screen resolution of 1.31579 µm/pixel. This step was carried out automatically for all frames covering one section. Each frame was addressed automatically and the number of stained cell nuclei per frame automatically counted and recorded. For the automatic detection of stained cell nuclei, threshold values can be set in AnalySIS for red, green and blue. No threshold was set for green. For red and blue, the threshold was set at 180 to 190, depending on the staining intensity for a given individual. Thus, for each pixel, values of red and blue were examined, if they were below the given threshold. This step resulted in valid and not valid pixels. A congregation of detected pixels was regarded as a stained cell nucleus if the covered region was between 4 and 100 µm^2. A stained nucleus had a size of about 20 µm^2. All 8 pixels surrounding one pixel were regarded adjacent to this pixel. Sometimes several cell nuclei lying close together were stained and occupied a somewhat larger region, therefore the upper threshold for this region was set at 100 µm^2. This value allowed for the detection of clustered stained cell nuclei, although they were counted as only one. On the other side, larger particles like dirt were still excluded.

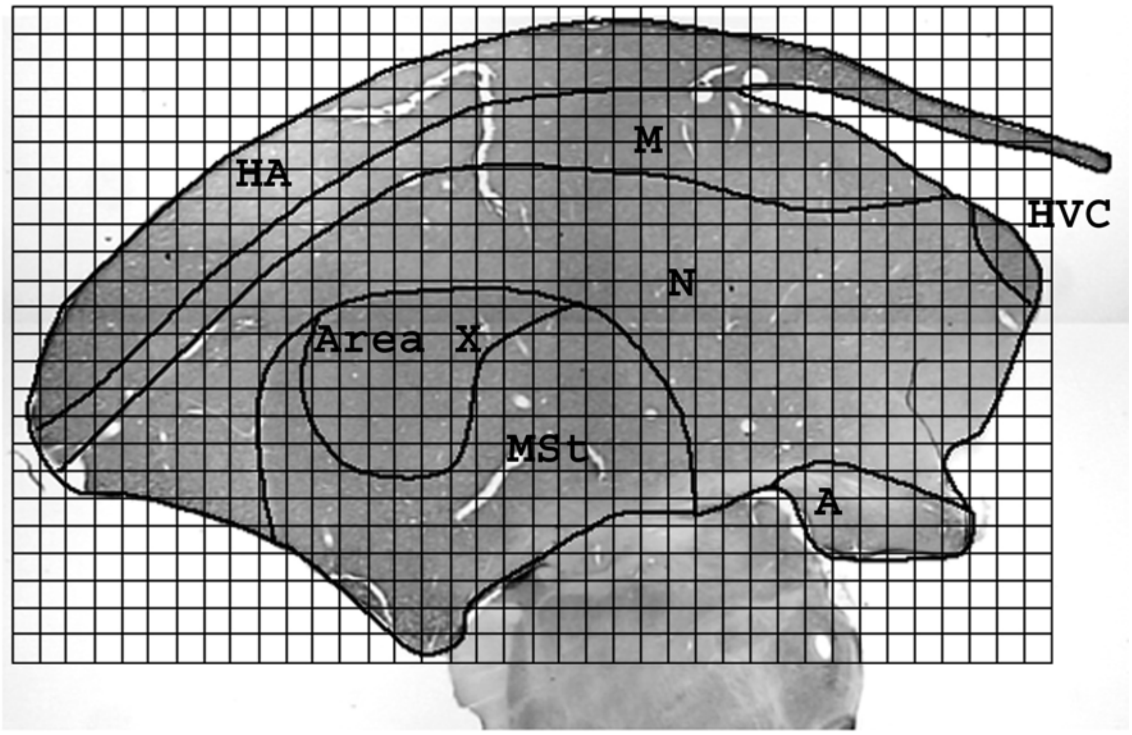


Figure 1 The grid represents the frames, each measuring 300 x 300 µm, for which the number of stained cell nuclei was automatically counted, and major subdivisions in the avian forebrain, as located in Red Bishop. The displayed parasagittal section was located 1.5 mm from median in the left hemisphere. Dorsal is up and frontal is left. HA, Hyperpallium apicale; M, Mesopallium; N, Nidopallium; MSt, Medial Striatum; A, Arcopallium.

2.4 Quantitative analysis of brain activity

For each individual, all sections were stained in the same batch. Because staining intensities as obtained by the immunocytochemistry varied between staining batches, thresholds for the detection of stained cell had to be adjusted for each individual separately. Consequently, in all sections of an individual, stained cell nuclei were counted with the same thresholds, but thresholds differed slightly between individuals. Therefore, the number of stained cell nuclei per frame was standardised for each individual separately to account for differences in staining intensity between individuals. Since the values for all frames were not normally distributed for each individual (Kolmogorov-Smirnov: all $p < 0.0005$), not a normalisation, but a transformation based on quartiles was performed. To standardise values, for each individual, the quartiles of the number of stained cell nuclei per 300 x 300 µm frame were calculated. Then the first quartile was set to -1, median was set to 0 and the third quartile was set to 1. Accordingly, values smaller than the median were rescaled relative to the distance given by the median and the first quartile. Values larger than the median were rescaled relative to the distance given by the median and the third quartile. This standardisation is comparable to a normalisation, only that median and quartiles instead of mean and standard deviation are used to rescale original values, and the scaling is different for values smaller than and larger than median, if the distance between the first quartile and median differs from the distance between median and the third quartile. After the standardisation, a value larger than 0 would indicate ZENK expression higher than median, and a value smaller than 0 would indicate ZENK expression smaller than median.

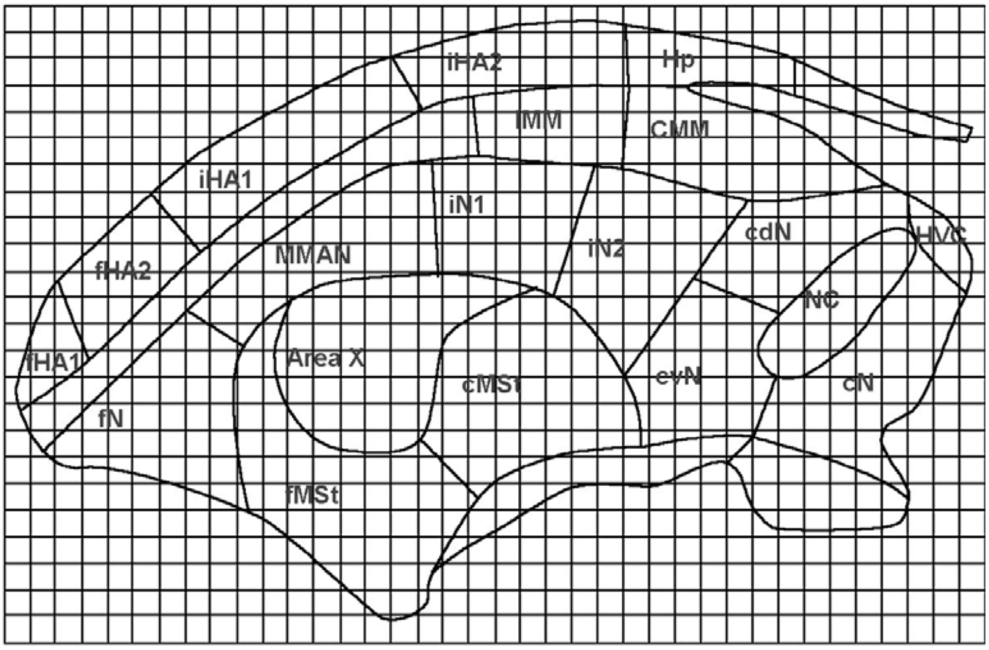


Figure 2 Exemplary scheme of regions of the forebrain for which ZENK expression was analysed. This scheme is based on the same section as Figure 1. The displayed section was located at 1.5 mm from median. Dorsal is up, frontal is left. One frame measured 300 x 300 µm.

For each analysed distance from median, a master template (Figure 2) was drawn and regions of interest were defined. Then for each analysed section, an individual template with the defined regions of interest for which ZENK expression should be determined was drawn according to the master template. Individual templates for every section were regarded necessary to take into account differences in the shape of individual brains. A total of 128 regions of interest were defined per hemisphere. Frames were labelled according to the region of interest they covered. Then for all regions of interest, the mean value of standardised ZENK expression was automatically computed from all frames labelled as falling into a particular region.

2.4.1 Combination of several regions

If a T-test comparing means of standardised values between control and experimental group for a given region was significant, this region was combined with adjacent, non-significant regions in neighbouring sections. The assumption giving reason for this procedure was that individually analysed regions lying adjacent to each other can be part of a functional unit showing similar ZENK expression in relation to nest-building behaviour. Because of the small sample size, not all analysed regions of such a functional unit might have shown significant differences between the experimental and control group. A combination of adjacent analysed regions could give evidence for such larger functional units. As an example, if there was a significant difference between building and control birds in the frontal nidopallium at 3.5 mm from median, the frontal nidopallium for 3.0 mm, 3.5 mm, and 4.0 mm were combined. The new combined region of interest should have maximum extensions of 2 mm in any axis, and this limit was already reached by the size of most initial regions of interest for each parasagittal section without the combination of several of these initial regions. Adjacent sections were only 500 µm apart, whereas most regions of interest had a dorsoventral and/or caudofrontal extension of at least 1500 µm (Figure 2). Therefore regions within the same section were not combined, because such a combination would have resulted in very large regions (see Jarvis & Mello (2000) for an example of sizes of avian forebrain nuclei). Since sections at 0.5 mm and 4.0 mm from median represented the medial and lateral limits of the analysed sections, for regions in these sections only regions in one directly adjacent section were available. In these cases corresponding regions in the next two sections were combined with the initial region. In other words, for a region in the 0.5 mm sections, the corresponding regions in the 1.0 mm and 1.5 mm sections were used for the combination of p-values, since no more medial section was available, and for a region in the 4.0 mm sections, the corresponding regions in the 3.5 mm and 3.0 mm sections were used for the combination of p-values, since no more lateral section was available.

2.5 Statistics

All statistical tests were performed with SPSS Software. All tests were two-tailed unless otherwise stated and α was set to 0.05. Because mean values for each analysed region based on standardised data of sampling frames were normally distributed (Kolmogorov-Smirnov: all $p > 0.10$), parametric tests were applied. The combination of several p-values followed Sokal & Rohlf (1995). For given Chi-square values and degrees of freedom, p-values were calculated with R language software. R computes parameters of the Chi-square distribution after Shea (1988). For general information on R, see e.g. Becker *et al.* (1988), Chambers *et al.* (1992) and Maindonald & Braun (2003).

3 Results

ZENK expression was analysed separately for each region of interest in each section. For each region, the mean for all analysed 300 x 300 µm frames falling into this region was calculated based on standardised values.

3.1 ZENK expression related to singing activity

To evaluate the general quality of the ZENK staining, I looked at ZENK expression related to singing activity in brain areas known to show ZENK expression during song production.

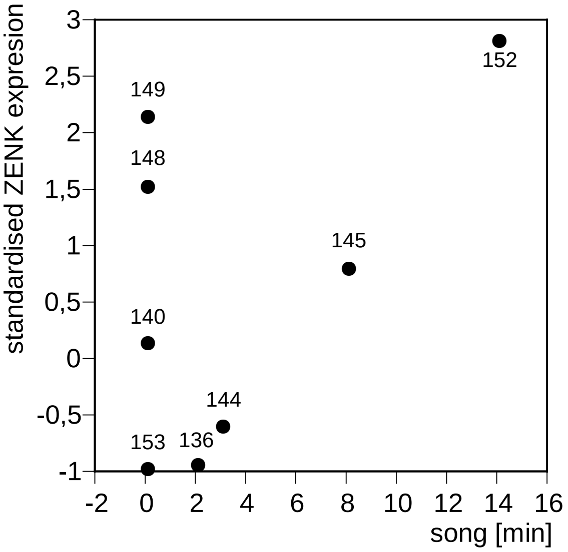


Figure 3 Standardised ZENK expression in relation to singing during the experiments in Area X 1.0 mm from median in the left hemisphere. The correlation is not significant. Note the high ZENK expression in birds that did not sing during the experiments. The numbers next to data points are birds' ring numbers.

Sample areas investigated for singing-induced ZENK expression were HVC, MMAN and Area X. For these areas, correlations were calculated between singing activity during the experiments and ZENK expression. A graphical example of ZENK expression after singing is given in Figure 3 for Area X at 1.0 mm from median in the left hemisphere. ZENK expression was not related to singing activity during the experiments in Area X, HVC, and MMAN (all $p > 0.06$).

3.2 ZENK protein expression regulated by building activity

For each of the 256 analysed regions of interest, mean standardised ZENK expression of all regions of interest for the experimental and control group were compared with T-tests. This test did not investigate if ZENK expression in a given region of interest was higher or smaller than in the rest of the forebrain, it tested, if standardised ZENK expression in defined regions of interest differed between building birds and non-building birds. Results are grouped according to forebrain subdivisions. Details for the statistical tests are given in Table 2. A graphical overview of the analysed regions and the significance of differences between experimental and control birds is given in Figure 4.

Table 2 Results of T-tests between nest-building and non-nest-building animals with regard to ZENK expression. Only results with $p < 0.5$ are displayed.

region	hemisphere	T	df	p
frontal HA part 2 1.0 mm from median	right	-2.825	5	0.037
frontal HA part 2 1.5 mm from median	right	-3.425	5	0.019
frontal N part 1 3.5 mm from median	left	-2.474	6	0.048
intermediate N 2.0 mm from median	left	3.509	5	0.017
NC 4.0 mm from median	right	-3.017	6	0.023
caudal M 2.0 mm from median	left	2.786	5	0.039
caudal MSt 0.5 mm from median	left	5.410	6	0.002

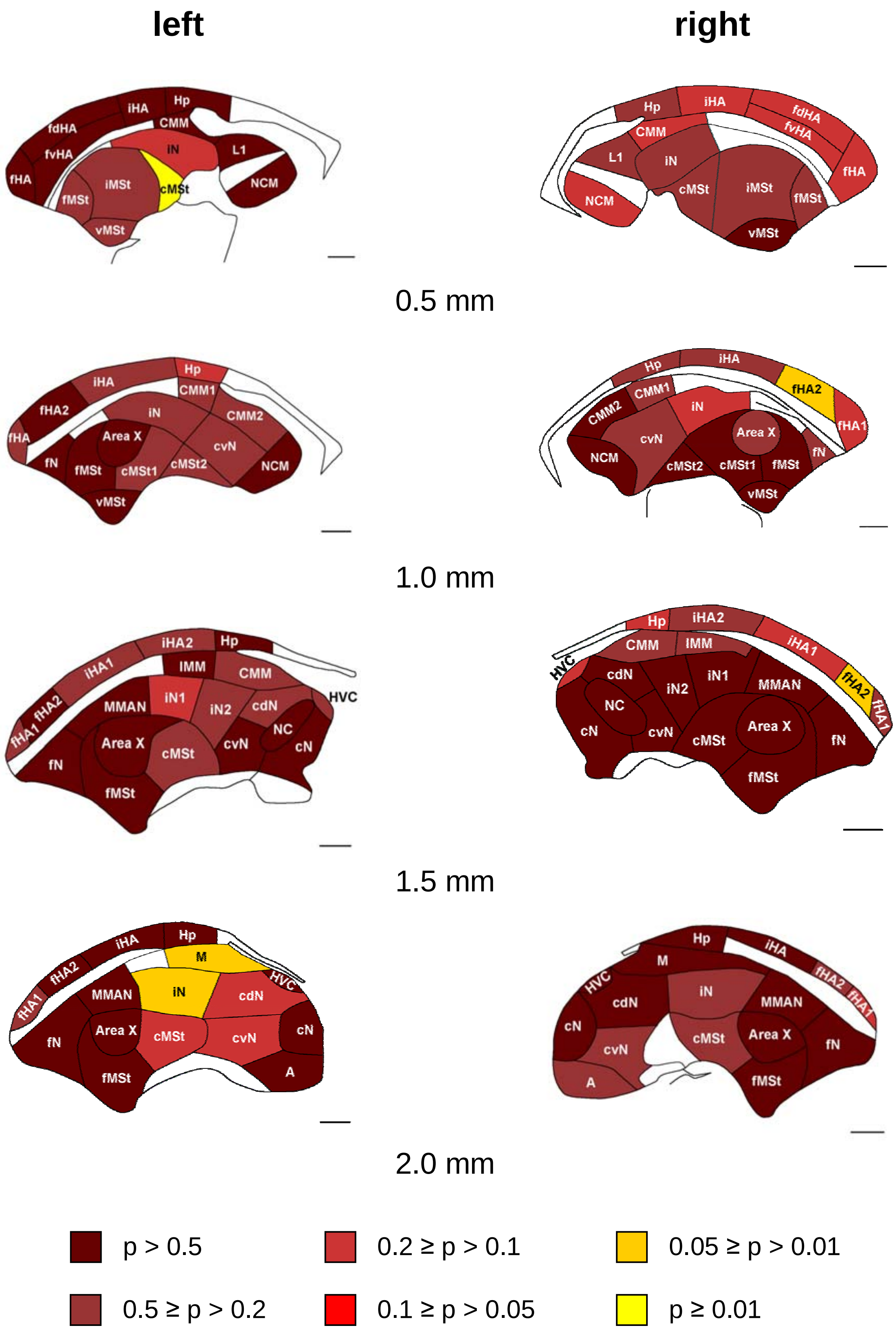


Figure 4 Graphical representation of differences between the experimental and control group in the analysed forebrain regions. Abbreviations: small letters indicate location (v = ventral, d = dorsal, f = frontal, c = caudal, i = intermediate). Capital letters follow conventional abbreviations (see Introduction). Scalebar is 1 mm.

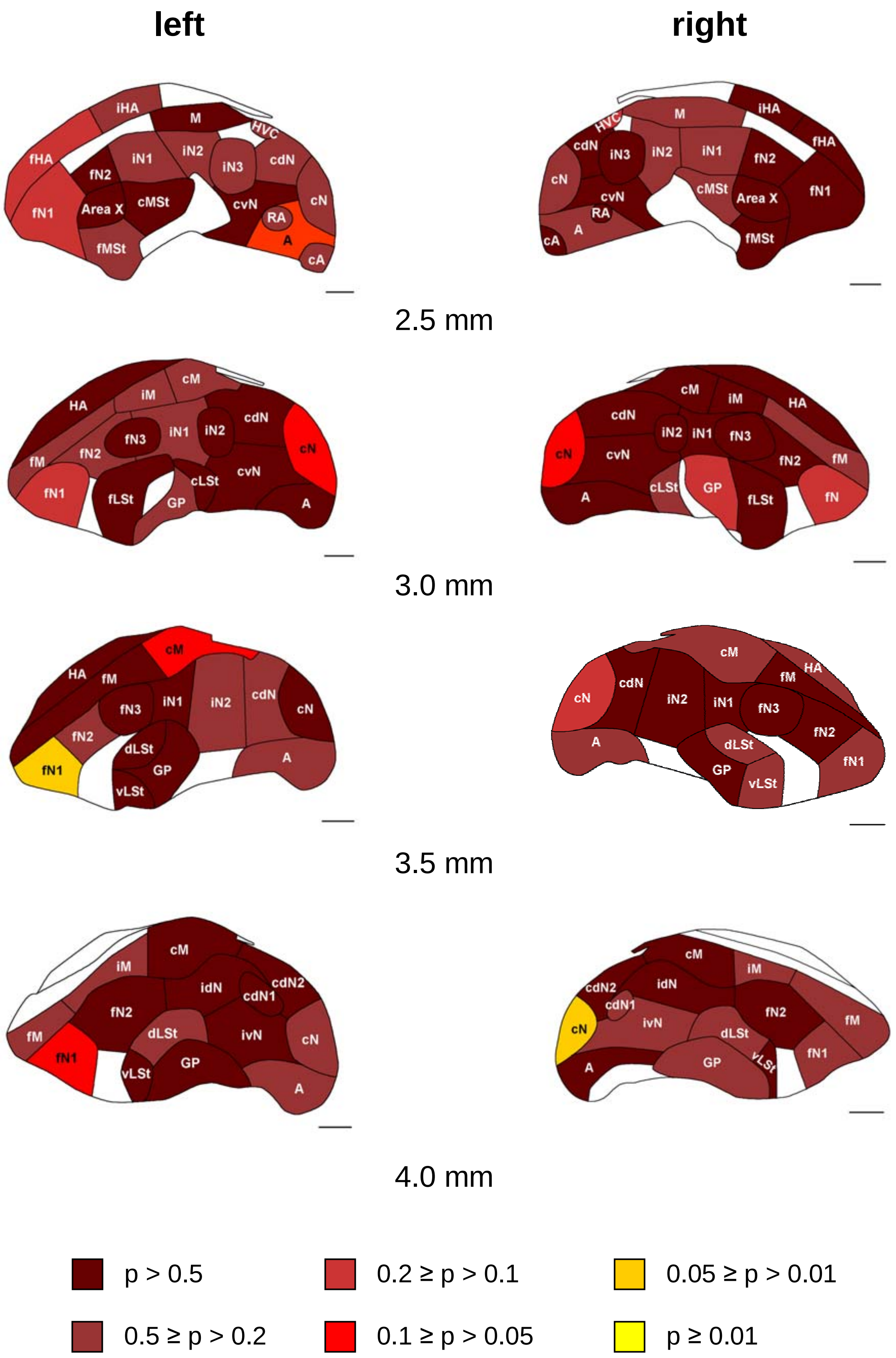


Figure 4 (continued)

3.2.1 Hyperpallium apicale

An increase in standardised ZENK expression after manipulation of nest-building material was observed in the frontal HA at 1.0 mm and 1.5 mm from median, only in the right hemisphere (Figure 5). ZENK expression in the right hemisphere at 1.0 mm and 1.5 mm from median in all individual brains is depicted in Figures 6 and 7.

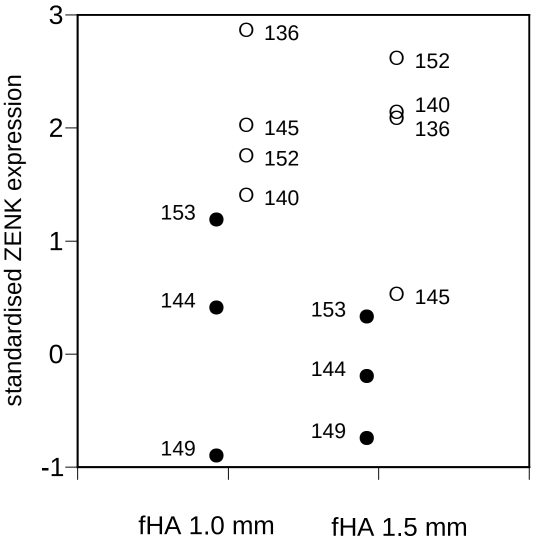
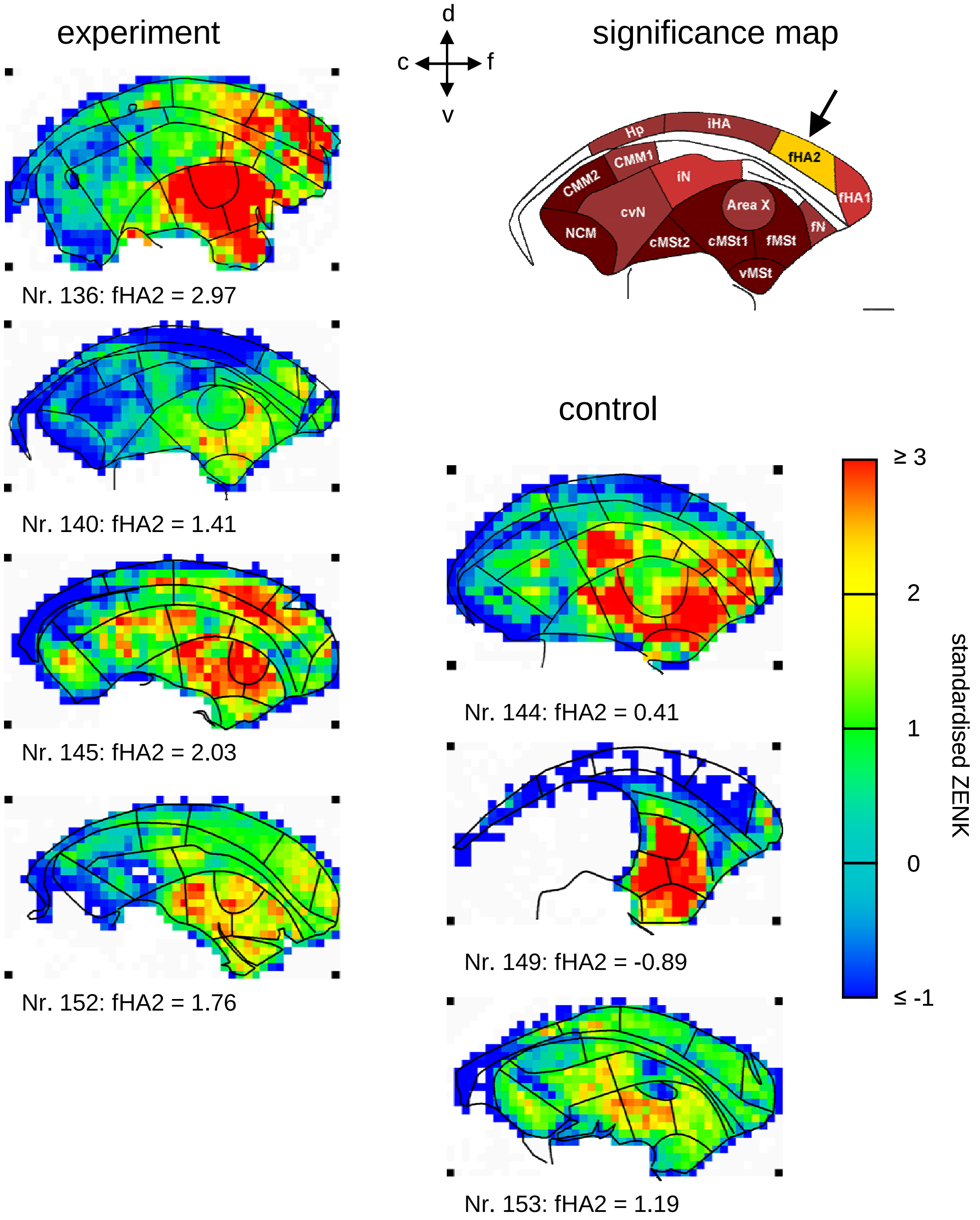


Figure 5 Standardised ZENK expression in the frontal hyperpallium apicale (fHA) at 1.0 mm and 1.5 mm from median in the right hemisphere was significantly higher in building than in control males. Solid circles: control; open circles: building. The numbers next to data points are birds’ ring numbers.



Nr. 136: fHA2 = 2.97

Nr. 140: fHA2 = 1.41

Nr. 145: fHA2 = 2.03

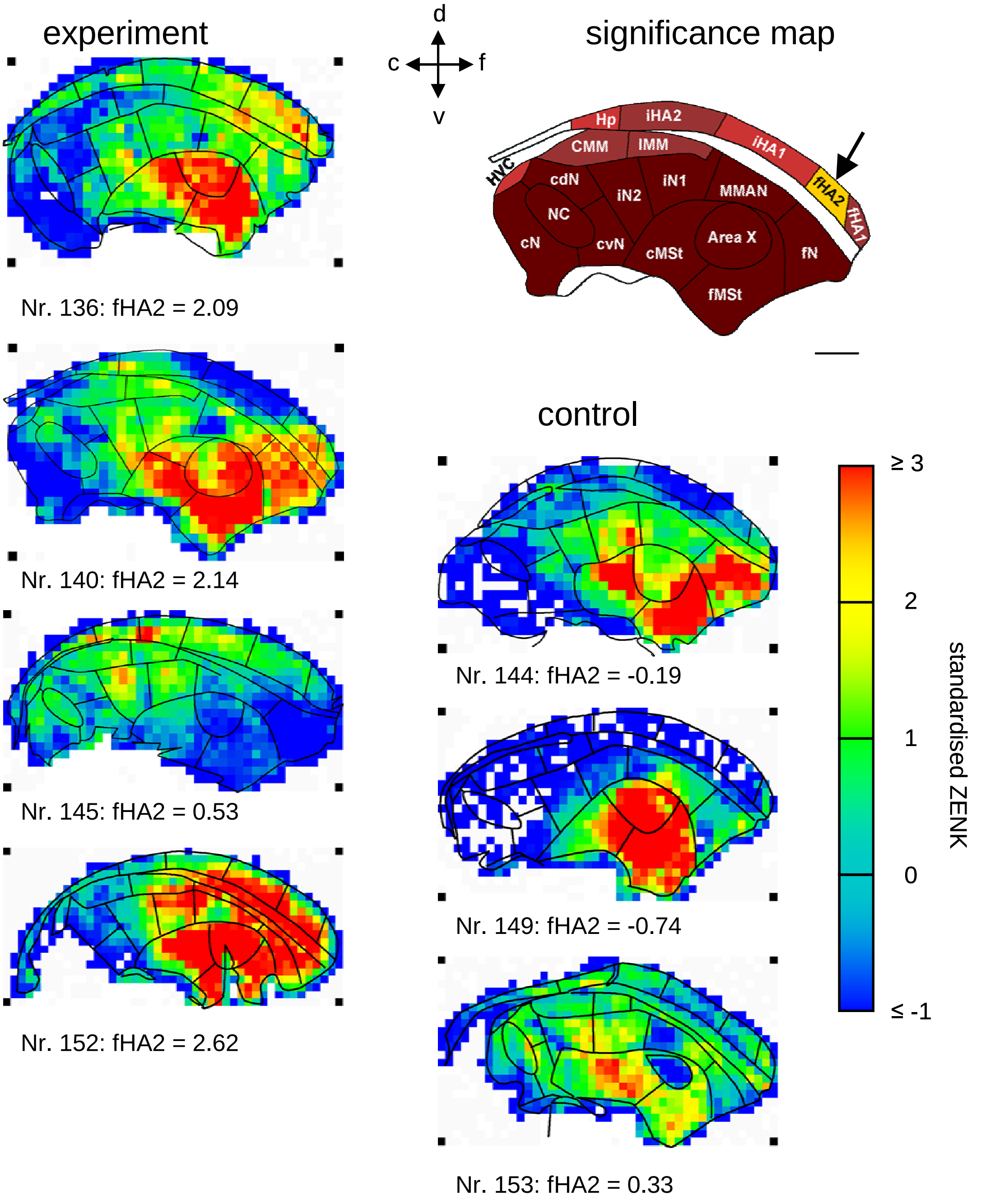
Nr. 152: fHA2 = 1.76

Nr. 144: fHA2 = 0.41

Nr. 149: fHA2 = -0.89

Nr. 153: fHA2 = 1.19

Figure 6 Graphical representation of ZENK activity in the sections 1.0 mm from median in the right hemisphere. White squares within the forebrain indicate that nothing could be detected. The colour coding of the significance map is the same as in Figure 4. Under every section are given the bird's ring number and the mean standardised value for fHA2, marked by an arrow in the significance map. For bird Nr. 149, the caudal part of the forebrain could not be processed.



Nr. 136: fHA2 = 2.09

Nr. 140: fHA2 = 2.14

Nr. 145: fHA2 = 0.53

Nr. 152: fHA2 = 2.62

Nr. 144: fHA2 = -0.19

Nr. 149: fHA2 = -0.74

Nr. 153: fHA2 = 0.33

Figure 7 Graphical representation of ZENK activity in the sections 1.5 mm from median in the right hemisphere. White squares within the forebrain indicate that nothing could be detected. The colour coding of the significance map is the same as in Figure 4. Under every section are given the bird's ring number and the mean standardised value for fHA2, marked by an arrow in the significance map.

3.2.2 Mesopallim

The mesopallium was divided into a maximum of three different regions per section. In the caudal mesopallium at 2.0 mm from median in the left hemisphere, ZENK expression in building birds was decreased compared to non-building birds

(Figure 8). ZENK expression at 2.0 mm from median in the left hemisphere in all individual brains is depicted in Figure 9.

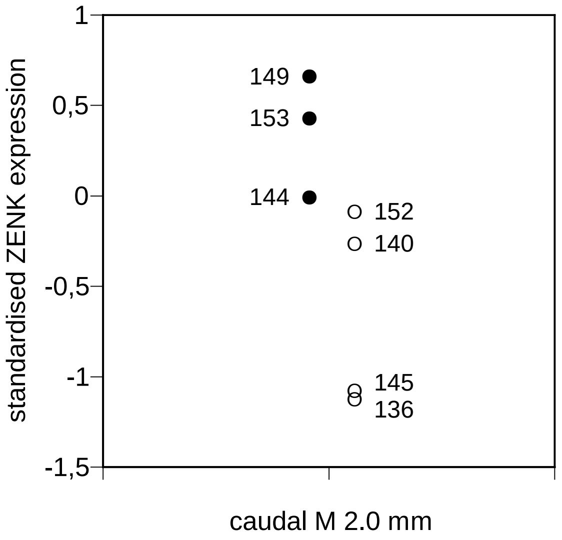
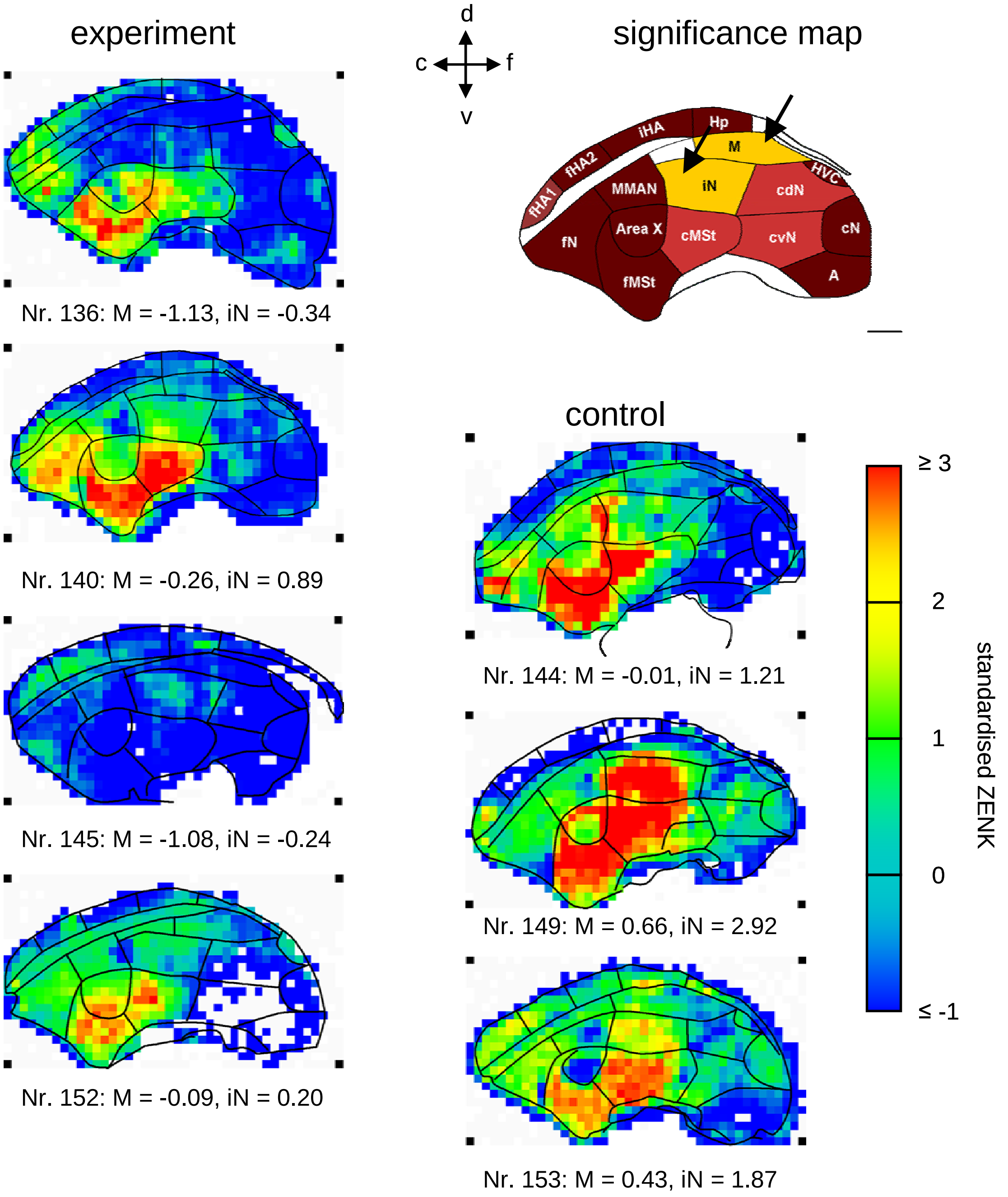


Figure 8 Standardised ZENK expression in the caudal mesopallium at 2.0 mm from median in the left hemisphere was significantly smaller in building than in control males. Solid circles: control; open circles: building. The numbers next to data points are birds' ring numbers.



Nr. 136: M = -1.13, iN = -0.34

Nr. 140: M = -0.26, iN = 0.89

Nr. 145: M = -1.08, iN = -0.24

Nr. 152: M = -0.09, iN = 0.20

Nr. 144: M = -0.01, iN = 1.21

Nr. 149: M = 0.66, iN = 2.92

Nr. 153: M = 0.43, iN = 1.87

Figure 9 Graphical representation of ZENK activity in the sections 2.0 mm from median in the left hemisphere. White squares within the forebrain indicate that nothing could be detected. The colour coding of the significance map is the same as in Figure 4. Under every section are given the bird's ring number and the mean standardised value for M and iN, marked by an arrow in the significance map.

3.2.3 Nidopallium

In three different regions of the nidopallium, an increase in ZENK expression after manipulation of nest-building material was observed (Figure 10). ZENK expression increased in the frontal nidopallium at 3.5 mm from median in left hemisphere. The second region was the intermediate nidopallium at 2.0 mm from median in the left hemisphere showing a decrease in ZENK expression. The third region was the caudal nidopallium at 4.0 mm from median in the right hemisphere with an increase in ZENK expression. See Figure 9 for ZENK expression in all individual brains at 2.0 mm from median in the left hemisphere. ZENK expression in all individual brains at 1.0 mm in the right hemisphere is depicted in Figure 11 and at 1.5 mm from median in the right hemisphere in Figure 12.

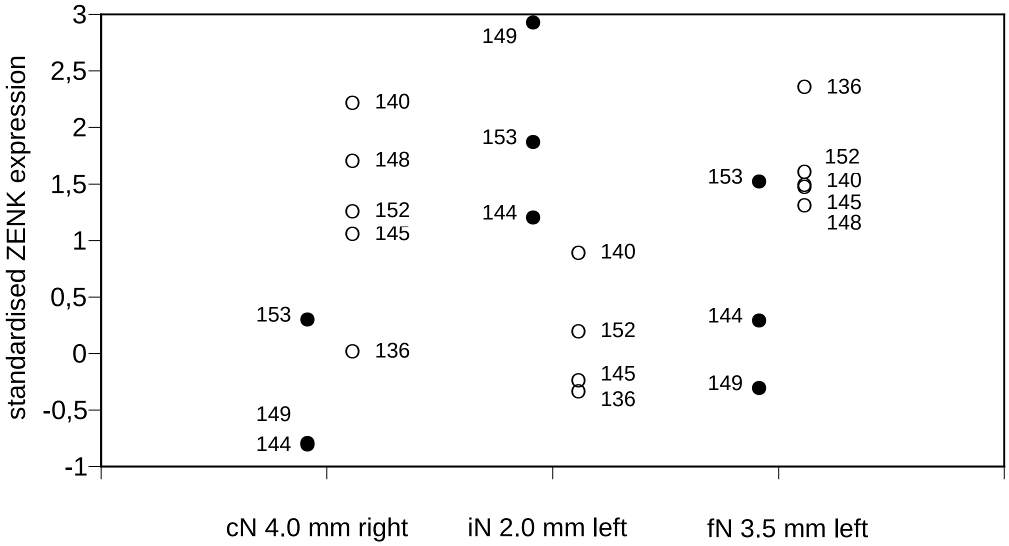
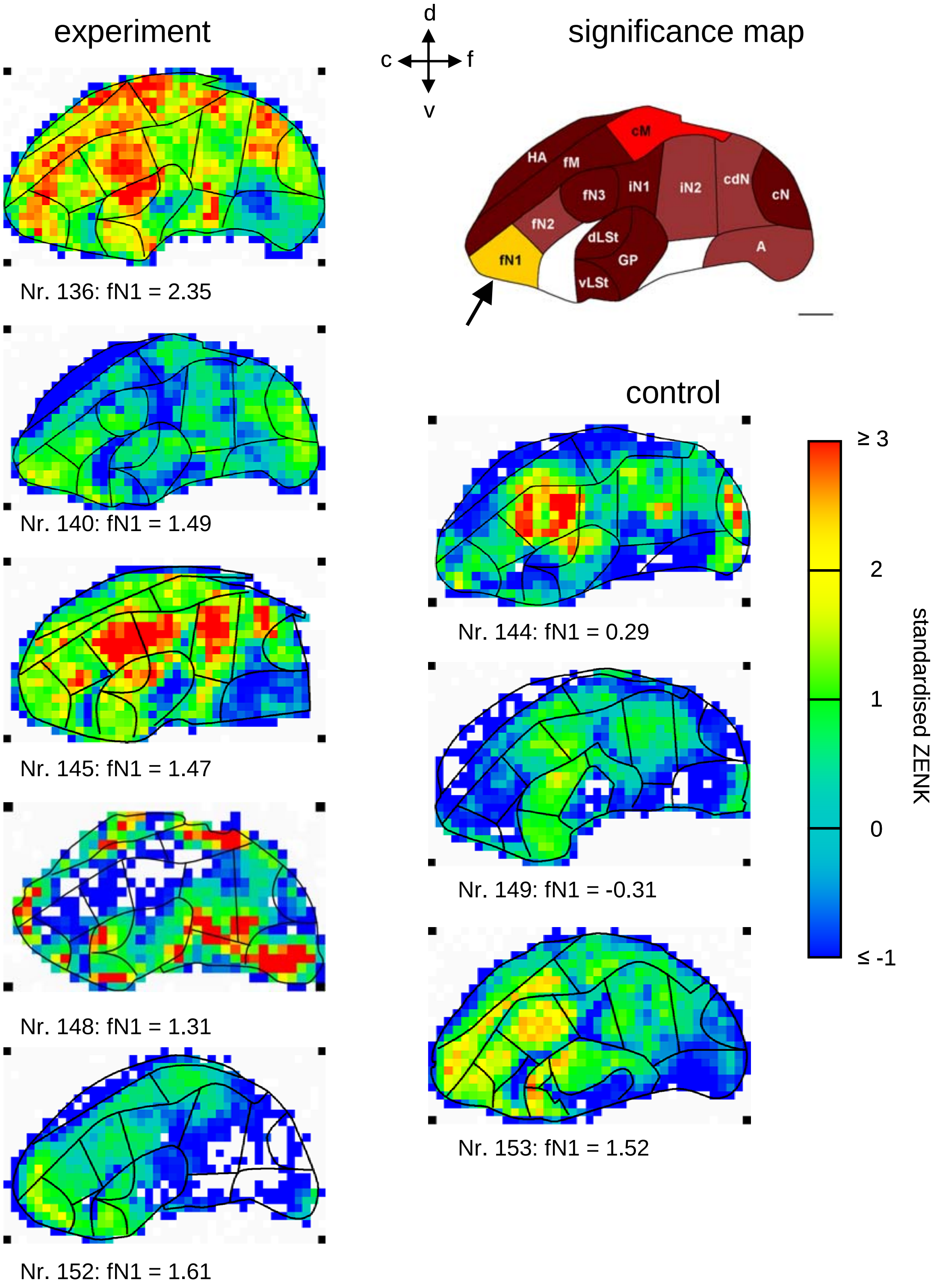


Figure 10 Standardised ZENK expression in three regions of the nidopallium with significant differences between building and control males. Solid circles: control; open circles: building. cN: caudal nidopallium; iN: intermediate nidopallium; fN: frontal nidopallium. Left or right indicates the hemisphere for which this difference between building and non-building males was found. The numbers next to data points are birds’ ring numbers.


experiment

significance map

control

Nr. 136: fN1 = 2.35

Nr. 140: fN1 = 1.49

Nr. 145: fN1 = 1.47

Nr. 148: fN1 = 1.31

Nr. 152: fN1 = 1.61

Nr. 144: fN1 = 0.29

Nr. 149: fN1 = -0.31

Nr. 153: fN1 = 1.52

Figure 11 Graphical representation of ZENK activity in the sections 3.5 mm from median in the left hemisphere. White squares within the forebrain indicate that nothing could be detected. The colour coding of the significance map is the same as in Figure 4. Under every section are given the bird’s ring number and the mean standardised value for fN1, marked by an arrow in the significance map.

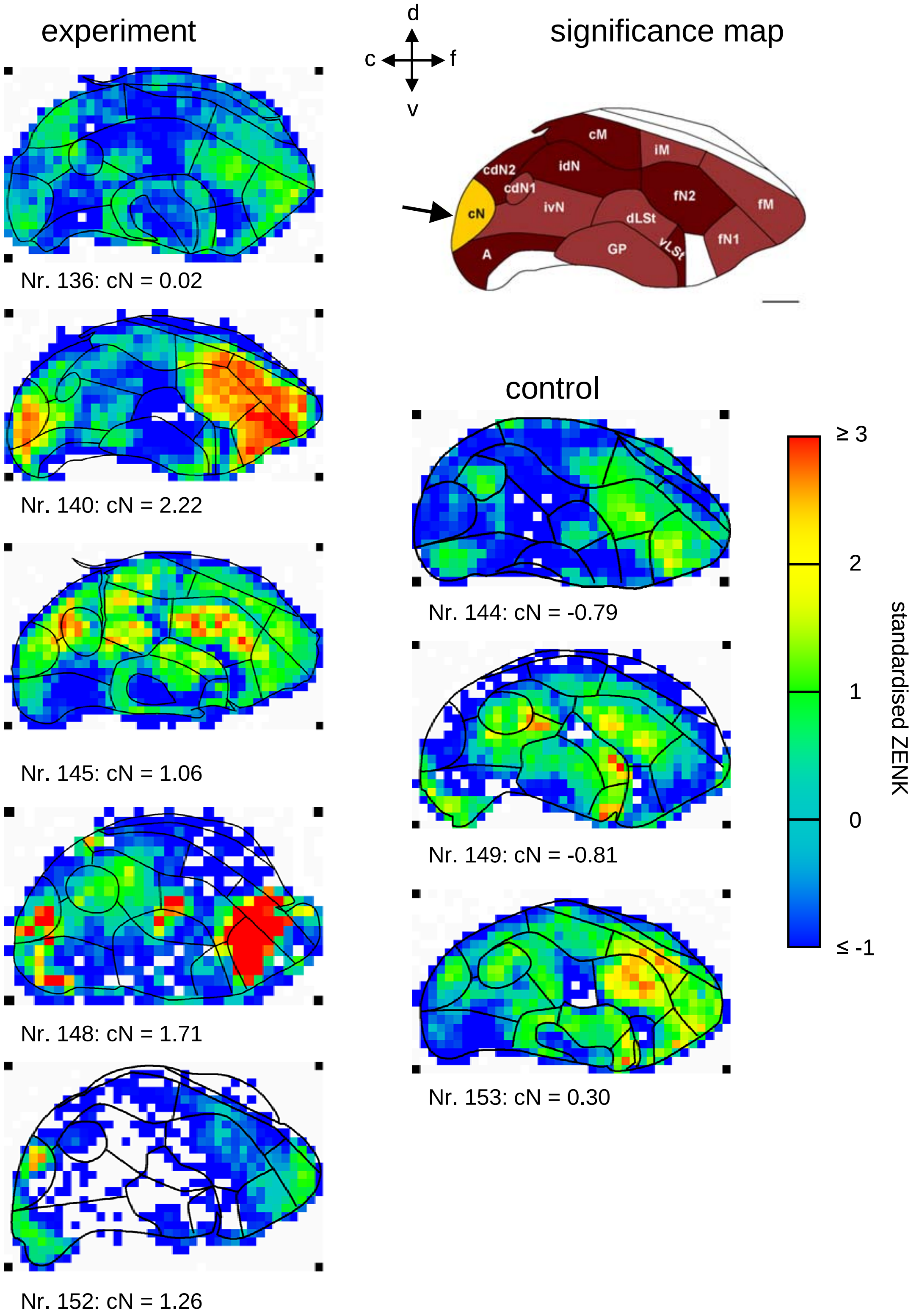


Figure 12 Graphical representation of ZENK activity in the sections 4.0 mm from median in the right hemisphere. White squares within the forebrain indicate that nothing could be detected. The colour coding of the significance map is the same as in Figure 4. Under every section are given the bird's ring number and the mean standardised value for cN, marked by an arrow in the significance map.

3.2.4 Medial striatum

ZENK expression was downregulated by nest-building behaviour in the caudal medial striatum at 0.5 mm from median only in the left hemisphere (Figure 13). No effect was found for the right hemisphere in this region. See Figure 14 for ZENK expression in all individual brains at 0.5 mm from median in the left hemisphere.

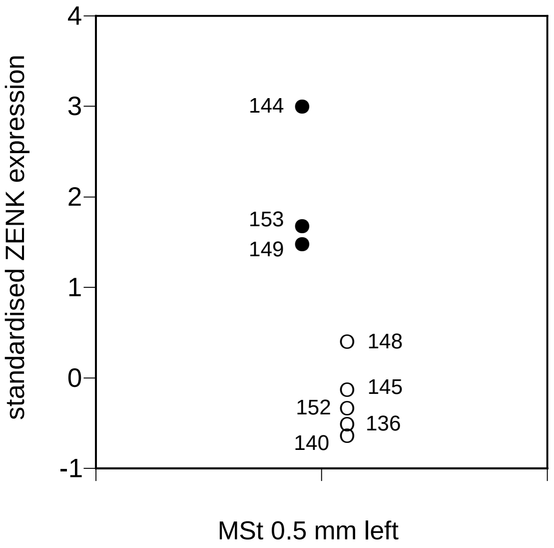


Figure 13 Standardised ZENK expression in the medial Striatum (MSt) at 0.5 mm from median in the left hemisphere with significant differences between building and control males. Solid circles: control; open circles: building. The numbers next to data points are birds' ring numbers.

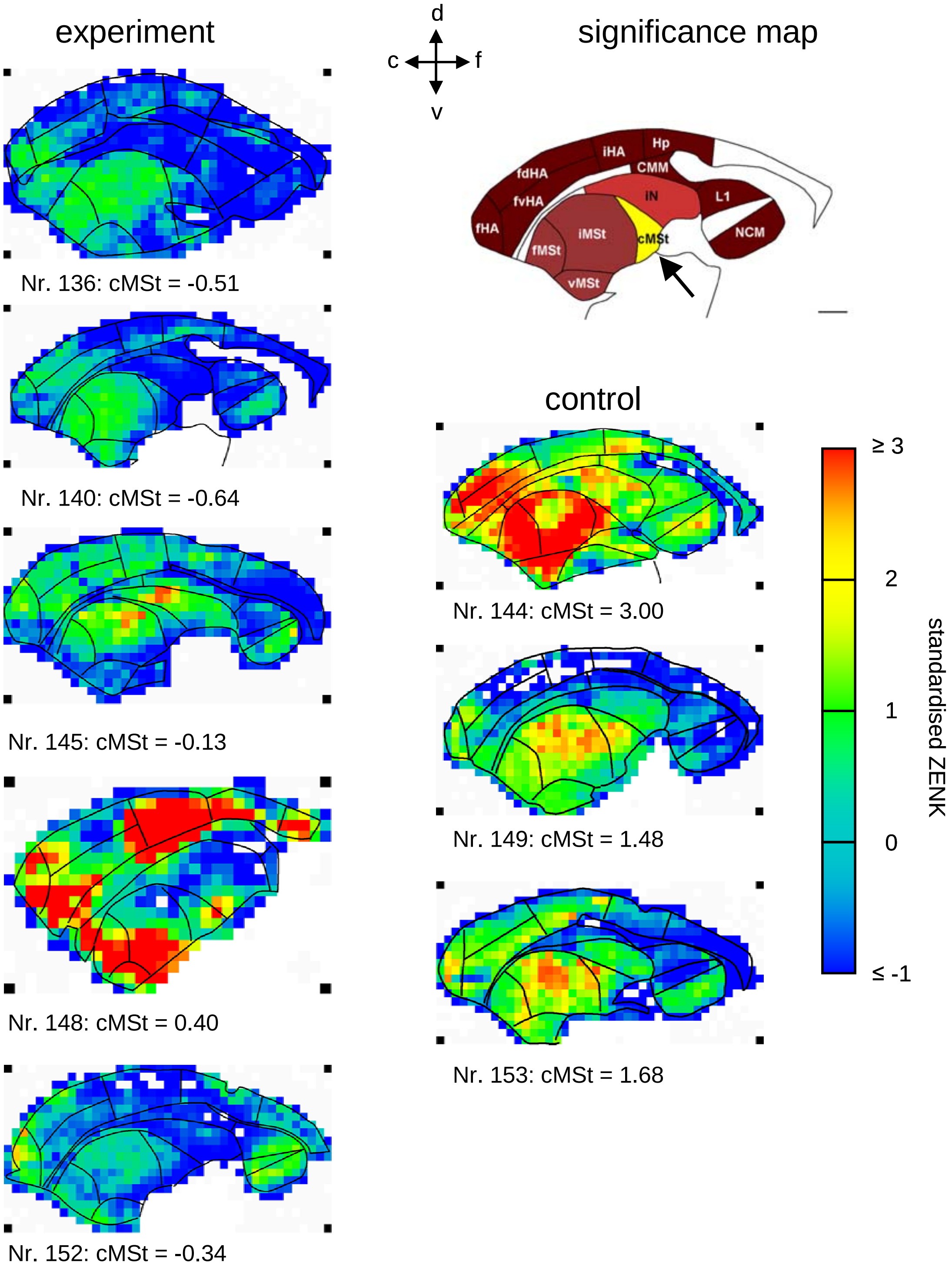


Figure 14 Graphical representation of ZENK activity in the sections 0.5 mm from median in the left hemisphere. White squares within the forebrain indicate that nothing could be detected. The colour coding of the significance map is the same as in Figure 4. Under every section are given the bird's ring number and the mean standardised value for cMSt, marked by an arrow in the significance map.

3.3 Combination of several adjacent regions

If the difference in ZENK expression for a region had a p-value <= 0.05, p-values of regions in the adjacent sections were combined. The only combination of adjacent regions not significant was the one for the caudal mesopallium from 1.5 mm to 2.5 mm in the left hemisphere. All other combined regions, the frontal hyperpallium apicale in the right hemisphere from 0.5 mm to 2.0 mm, the caudal nidopallium in the right hemisphere from 3.0 mm to 4.0 mm, the intermediate nidopallium in the left hemisphere from 1.5 mm to 2.5 mm, the frontal nidopallium in the left hemisphere from 3.0 mm to 4.0 mm, and the caudal medial striatum in the left hemisphere from 0.5 mm to 1.5 mm were significant (Table 3), that is in these regions, levels of ZENK expression in building birds were different from levels of ZENK expression in non-building birds.

Table 3 Regions of interest adjacent to a region with $p < 0.05$ in T-tests between experimental and control group were combined with this region.

combined region	section	p	chi-square	df	combined p
frontal HA	0.5 rostrodorsal HA	0.101	25.722	10	0.0041
right hemisphere	0.5 rostroventral HA	0.135			
	1.0 frontal HA	0.037			
	1.5 frontal HA	0.019			
	2.0 frontal HA	0.271			
caudal M	1.5 caudomedial M	0.279	9.266	6	0.1592
left hemisphere	2.0 caudomedial M	0.039			
	2.5 caudomedial M	0.894			
frontal nidopallium	3.0 frontal nidopallium	0.147	14.746	6	0.0223
left hemisphere	3.5 frontal nidopallium	0.048			
	4.0 frontal nidopallium	0.089			
intermediate	1.5 intermediate N	0.122	13.958	6	0.0301
nidopallium	2.0 intermediate N	0.017			
left hemisphere	2.5 intermediate N	0.449			
caudal nidopallium	3.0 caudal N	0.088	16.160	6	0.0129
right hemisphere	3.5 caudal N	0.153			
	4.0 caudal N	0.023			
caudal medial	0.5 caudal MSt	0.002	17.417	6	0.0079
striatum	1.0 caudal MSt	0.280			
left hemisphere	1.5 caudal MSt	0.295			

3.4 Differences between hemispheres

For all above mentioned regions with a marked difference in ZENK expression, these effects were limited to one hemisphere only, but not always the same hemisphere. To test the validity of these differences between hemispheres, differences between the left and right hemisphere in ZENK expression were calculated for each region with ZENK expression changing with nest-building behaviour. With these differences, T-tests were performed. If ZENK expression gets altered in only one hemisphere during nest-building, the values for the differences should not deviate from zero for the control group, contrary to the experimental group. If the above results are due to statistical errors, and the change in ZENK expression is equal for both hemispheres (not existing, down- or upregulated), then the differences between hemispheres should be close to zero for both the control and the experimental group. If ZENK expression after nest-building behaviour increased only in one hemisphere, the difference in ZENK expression between hemispheres should be larger for the experimental group than for the control group if ZENK expression for the region in the hemisphere showing no effect is subtracted from ZENK expression for the region in the hemisphere showing a positive effect. Vice versa, the difference between hemispheres should become negative if ZENK expression is downregulated by nest-building behaviour and ZENK expression for the region in the hemisphere showing no effect is subtracted from ZENK expression for the region in the hemisphere showing a positive effect. To facilitate comparisons of the results between the analysed regions, ZENK expression for the region in the hemisphere showing no effect was subtracted from ZENK expression for the region in the hemisphere showing an effect according to Table 3. Results of all tests are given in Table 4 and illustrated in Figure 15.

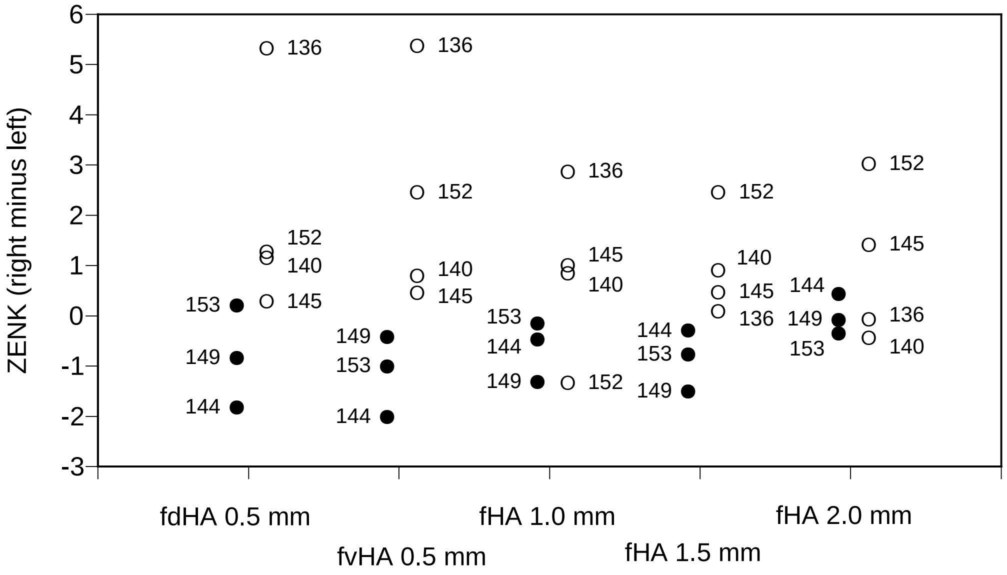


Figure 15 The differences in standardised ZENK expression between right and left hemisphere in the frontal HA from 0.5 mm to 2.0 mm from median were larger for building animals than the control group when p-values for all areas were combined. Solid circles: control; open circles: building. fdHa: rostrodorsal HA; fvHA: rostroventral HA, fHA: frontal HA.

Differences in ZENK expression between regions were again combined as in Table 3, with the exception of the caudomedial mesopallium, where the combination across several sections did not reveal any change in ZENK expression after nest-building. Therefore for the caudomedial mesopallium only the section 2.0 mm from median was analysed for differences between experimental and control group in their differences between hemispheres in ZENK expression. The difference in ZENK expression between hemispheres did not differ between experimental and control group in the caudomedial mesopallium at 2.0 mm from median (T = 1.308, df = 5, p = 0.248). After combining p-values for adjacent sections, the difference in ZENK expression between hemispheres was affected by nest-building only in the frontal HA from 0.5 mm to 2.0 mm from median (Figure 15), where it was larger in building than in not building animals (Table 4).

Table 4 Comparison of experimental and control group in their differences in ZENK expression between hemispheres for regions with modulation of ZENK expression after nest-building behaviour. P-values for regions in adjacent sections were combined.

combined region	section	p	chi-square	df	combined p
frontal HA	0.5 rostrodorsal HA	0.104	21.675	10	0.0168
	0.5 rostroventral HA	0.057			
	1.0 frontal HA	0.219			
	1.5 frontal HA	0.043			
	2.0 frontal HA	0.352			
caudal nidopallium	3.0 caudal N	0.371	4.0765	6	0.6663
	3.5 caudal N	0.422			
	4.0 caudal N	0.832			
intermediate nidopallium	1.5 intermediate N	0.27	5.7187	6	0.4554
	2.0 intermediate N	0.263			
	2.5 intermediate N	0.807			
frontal nidopallium	3.0 frontal nidopallium	0.439	4.0356	6	0.6718
	3.5 frontal nidopallium	0.728			
	4.0 frontal nidopallium	0.416			
caudal medial striatum	0.5 caudal MSt	0.043	8.9360	6	0.1772
	1.0 caudal MSt	0.55			
	1.5 caudal MSt	0.485			

4 Discussion

This study attempted to identify forebrain areas involved in the sensory and motor control of nest-building in a weaverbird by means of the detection of differences in ZENK protein expression. Since it is not known which regions could possibly be involved, an approach was chosen that allowed for a screening of major parts of the avian forebrain. The avian forebrain has been well investigated (Reiner *et al.* 2004, and references therein) and can be divided into six major subdivisions, the hyperpallium, mesopallium, nidopallium, arcopallium, and medial and lateral striatum. According to Jarvis & Mello (2000), an avian forebrain pathway for sensory and motor control of any complex behaviour should consist of regions in all these major subdivisions. A quick overview of the general function of each major subdivision shall be given, but a detailed discussion of the organisation of the avian forebrain is regarded inappropriate here. The general function of the hyperpallium is the processing of visual and somatosensory input (Reiner *et al.* 2004). The three regions well known in the mesopallium are part of the vocal circuit (oval nucleus), the auditory circuit (caudal medial mesopallium), and are involved in learning and imprinting (intermediate medial mesopallium). The nidopallium receives general sensory input. Within it, three major areas with extratelencephalic input are known, namely field L (auditory input), entopallium (visual input), and nucleus basorostralis pallii (input from nervus trigeminus; Reiner *et al.* 2004). Other parts of the nidopallium further process this sensory input, e.g. the NCM, LMAN and MMAN for auditory perception (review in Brainhard & Doupe 2002) and the NCL as working memory and for triggering motor commands (Diekamp, Kalt, Güntürkün 2002). The NCM in particular is well investigated in its role for auditory processing, song learning and control (e.g. Kruse *et al.* 2000, Mello *et al.* 1992, Mello & Clayton 1994, Mello *et al.* 1995, Jarvis *et al.* 2000). The arcopallium possesses mainly somatic features (review in Reiner *et al.* 2004). The medial and lateral striatum are regarded as the striatal part of avian dorsal basal ganglia (Reiner *et al.* 2004). Within the medial striatum, Area X is well investigated in regard to its context-dependent induction of ZENK by singing (Jarvis & Nottebohm 1997, Jarvis *et al.* 1998). The globus pallidus is of interest with respect to nest-building because of its motor output connections (Reiner *et al.* 2004).

In the present study, forebrain regions involved in sensory and motor control of nest-building were identified by ZENK protein immunoreactivity of cell nuclei. Immediate Early Genes are widely used markers for neural activity in the brain (Long & Salbaum 1998) and were named Immediate Early Genes because of their fast response to external stimuli or to changes in a behavioural state. The time course of this response with a rapid increase and a slower decrease inspired the term genomic action potential (Clayton 2000) analogous to the electrophysiological action potential, although on a very different time scale. The expression of IEGs is not restricted to novel contexts and memory formation, but rather associated with contexts in some way important for the organism (Clayton 2000).

Zenk mRNA levels were not only changed after singing, but correlated both positively and negatively with the number of song bouts in Zebra Finches (*Taeniopygia guttata*; Jarvis & Nottebohm 1997, Jarvis *et al.* 1998) and several species of hummingbirds (Jarvis *et al.* 2000). Sadananda & Bischof (2004) were able to distinguish four different treatments based on the number of Fos immunoreactive cell nuclei in experiments with Zebra Finches on sexual imprinting. Terpstra *et al.* (2004) found a correlation between ZENK protein expression and strength of song learning in the NCM of Zebra Finches. ZENK protein expression also correlated positively with the number of different syllables in the NCM and CMM of budgerigars (*Melopsittacus undulatus*) (Eda-Fujiwara *et al.* 2003) and in the NCM of Zebra Finches (Bolhuis, Zijlstra *et al.* 2000). If there is a correlation between stimulus intensity and the number of neurons showing IEG expression in a defined brain area, it could be possible that this effect is actually caused by several slightly different stimuli, the number of which representing stimulus intensity. On the level of individual neurons, there is only an all-or-none response in IEG expression (Shin *et al.*1990, Worley *et al.* 1993). In a given brain region with well defined function, different neurons may be activated by slightly different stimuli (Clayton 2000). A higher number of slightly different stimuli would thus lead to a higher number of neurons showing IEG expression in a given brain region. This can explain why only a few neurons in a given area show IEG expression in response to only few stimuli and more neurons get activated with more, different stimuli. This interpretation is in accordance with Bolhuis, Zijlstra *et al.* (2000), Eda-Fujiwara *et al.* 2003, Jarvis & Nottebohm (1997), Jarvis *et al.* (1998), Jarvis *et al.* (2000), Sadananda & Bischof (2004), and Terpstra *et al.* (2004). The habituation of IEG expression to repeated stimuli is well known (e.g. Clayton 2000, Mello *et al.* 1995, Stripling 1997). On the other hand, an increase in stimulus variety, i.e. the presentation of more stimuli that are similar but not equal, will lead to an increase in IEG expression. Transferred to nest-building, the act of waving with a fibre can be broken down into a variety of different motor patterns. First of all, there is an obvious difference between weaving a fibre around a twig and weaving a fibre in the mesh wire of an aviary. Weaving a fibre around a twig can again be broken down into several distinct motor patterns like holding the fibre with a foot, pushing the fibre around a twig, letting go the fibre in order to pick up the fibre again on the other side of the twig. The point being, the more fibres a weaverbird manipulates, the more motor patterns get executed, however subtle in their differences. This increase in variety should lead to a higher number of activated neurons and facilitate the identification of regions within the forebrain involved in sensory and motor control of nest-building, when yearling male weaverbirds are allowed to manipulate several fibres.

A number of brain areas are known to be involved in learning and sensorimotor control of a variety of behavioural contexts with a corresponding change of IEG expression. The hippocampal formation in birds is known to be involved in memory formation, e.g. in the contexts of food storing (Healy & Krebs 1993) and sexual imprinting (Lieshoff *et al.* 2004). In chicks, Fos activity was higher in trained than in control animals in an imprinting experiment (McCabe & Horn 1994) in the dorsolateral hippocampus. In a food hoarding task with Black-capped Chickadees (*Poecile atricapillus*), ZENK expression in the hippocampus was positively linked to the accuracy of cache retrieval (Smulders & DeVoogd 2000). The hyperpallium apicale had higher Fos expression during first courtship than in controls in Zebra Finches (Sadananda & Bischof 2002). The intermediate medial part of the mesopallium (IMM) is related to learning, not motor activity, in domestic chicks (*Gallus domesticus*), and good learners showed more Fos activity in the IMM in an imprinting task (McCabe & Horn 1994). The lateral and medial magnocellular nucleus of the anterior nidopallium (LMAN and MMAN, respectively) showed an increase in ZENK expression during singing (Jarvis & Nottebohm 1997). The lateral nidopallium had higher Fos expression during first courtship than in controls (Sadananda & Bischof 2002). All these areas are not exclusive to songbirds and are not exclusively involved in the control of singing activity. Brain areas that are exclusively found in songbirds and are related to song learning, perception or production (Brenowitz

1997, Jarvis *et al.* 2000) were not expected to be involved in controlling nest-building behaviour, but ZENK expression in these areas was also determined, since it came at negligible additional costs.

Both sexual imprinting (review in Bischof 2003) and filial imprinting (review in Horn 1998) are not regarded as suitable for a comparison with nest-building, because they only comprise sensory learning and no learning of motor patterns. In general, singing in passerines and nest-building in weaverbirds have several features in common. For both behavioural patterns, (i) the first year of life is crucial for the learning process, (ii) both patterns involve sensory input processing and (iii) the development of both patterns requires learning of complex sequential motor actions. In their early stages, songbirds produce plastic song and yearling male weaverbirds build nests that differ in their appearance from nests of adult birds. In both cases, the sequential motor patterns are still not perfect and need to be refined by comparison with an internally stored master template, the learned species-typical song and the probably inherited (Collias EC & Collias NE 1964) species-typical nest shape, respectively. A conclusion from a comparison between singing in songbirds and nest-building in weaverbirds would be that some regions in the forebrain are specialised on sensory or motor control of nest-building.

4.1 General experimental design

In most birds singing occurs in bouts and continuously during at least 30 minutes (Catchpole & Slater 1995), whereas nest-building behaviour even in adult red bishops never occurred for longer than 10 minutes (pers obs). Changes in IEG expression can be detected after acoustic stimulus presentation as short as 3 min (Scheich & Zuschratter 1995) or even 2 s (Kruse *et al.* 2000), but for optimal IEG expression, a stimulus or behaviour should be present for about 30 min (Jarvis & Nottebohm 1997). In the experiments presented here, yearling male Red Bishops did not engage continuously in nest-building behaviour for longer than 2 min, but showed a variety of behavioural patterns, which probably rendered the detection of ZENK expression linked to nest-building difficult. In pilot experiments, birds did not show nest-building behaviour when kept in social isolation, and experiments had to be conducted in a room with other birds that were not visible, but audible. Because of confounding but necessary stimuli (conspecifics), this setup increased the difficulty of identifying forebrain regions involved in the control of nest-building. Therefore the above mentioned regions should be regarded as a first step encouraging further research, because it is reasonable to assume that more forebrain regions than the ones detected in this study are involved in sensory and motor control of nest-building in weaverbirds. On the other side, the identified forebrain regions should be regarded with care and investigated further to confirm and better describe their role in controlling nest-building behaviour. A total of 256 different forebrain regions were tested for differences in ZENK expression between experimental and control animals. With this large number, it is possible that some differences are significant or also non-significant purely by chance, also demanding further confirmation by a more detailed investigation of these regions. Therefore the above mentioned regions should be regarded as a first step encouraging further research, because it is reasonable to assume that more forebrain regions than the ones detected in this study are involved in sensory and motor control of nest-building in weaverbirds. A possible modification of the experimental setup would replace actual conspecifics in the same room by playbacks, by which this stimulus that also modulates IEG expression in the avian forebrain could be kept constant. Apart from these considerations on the experimental setup, any future experiments on forebrain activity in relation to nest-building behaviour should be conducted with a slightly larger sample size to reduce the possibility of type II statistical errors. In general, brain activity and IEG expression seem to increase over major parts of the forebrain when songbirds are hearing song or singing themselves or both (Jarvis & Nottebohm 1997), although IEG regulation dependent on singing and hearing is only shown for well defined nuclei (the auditory forebrain pathway; review in Brainhard & Doupe (2002)), whereas the remaining parts of the forebrain do not seem to be involved in singing or hearing, despite a slight general increase in IEG activity, judged by visual inspection. Statistically spoken, the null hypothesis that these major parts of the forebrain did not show a change in IEG expression could not be rejected, and it can not be excluded that other parts of the forebrain do show changes in IEG expression, if only minute.

With these considerations on the experimental setup and the validity of the obtained results in mind, the found differences in ZENK expression are discussed in the following.

4.2 ZENK expression induced by singing

A number of forebrain areas are known to be specialised on singing and hearing (review in Brainhard & Doupe 2002) with corresponding IEG expression (Jarvis *et al.* 2002). In the study presented here, no correlation was apparent between ZENK expression in Area X, HVC, or MMAN and singing activity during the experiments. ZENK expression in these areas could be expected in the experimental situation when the individual was singing (Jarvis & Nottebohm 1997) in a solo context (Jarvis *et al.* 1998). Although experiments were conducted in the morning, it can not be excluded that the birds were singing prior to the experiments, before behavioural data were recorded. Thus, ZENK expression in relation to singing activity could be high for individuals that did not sing during the experiment. Alternatively, if birds sang before the experiments, birds might have been perfused after ZENK expression peaked in response to singing. In this case, ZENK protein expression in relation to singing activity prior to experiments would have been declining at the time of perfusion, and impeded the detection of ZENK expression related to singing during the experiments. The data distribution for Area X at 1.0 mm from median in the left hemisphere lets suggest that birds that did not sing during the experiments were singing prior to experiments, causing increased levels of ZENK expression. Additionally, only two individuals were singing often during the experiments, two other individuals showed only little singing activity, and the remaining four individuals were not singing at all. Further on, with 4 birds not singing at all and only 2 birds singing at least for 8 min during the experiments, the data presented here were not suitable for an analysis of singing-related ZENK expression. Therefore the missing confirmation of singing-related ZENK expression in Area X, HVC, and MMAN does not question the validity of the main analysis presented here.

4.3 ZENK expression induced by nest-building

The avian forebrain can be morphologically and functionally divided into five large subdivisions, the hyperpallium, mesopallium, nidopallium, arcopallium, and striatum, from dorsolateral to ventromedial (Reiner *et al.* 2004). In the following, results are discussed in this order.

4.3.1 Hyperpallium

This region processes general visual and somatosensory information received from parts of the thalamus (Reiner *et al.* 2004). Up to 4 regions per section were analysed in the hyperpallium apicale. The largest subdivision showing increased ZENK expression after nest-building activity was the frontal hyperpallium apicale from 0.5 mm to 2.0 mm in the right hemisphere. This area is covering the area of the hyperpallium apicale investigated by McCabe & Horn (1994), who found less Fos in good learners than in poor learners or control in chicks in an imprinting task (McCabe & Horn 1994). Fos and ZENK are known to differ in their expression patterns (Ball & Balthazart 2001). Therefore the differences in Fos expression in chicks and ZENK expression in Red Bishops in the frontal median hyperpallim apicale do not have to be contradictory.

4.3.2 Mesopallium

The combination of adjacent sections did not reveal a larger region with a decrease in ZENK expression in the caudal mesopallium after nest-building, and the difference in ZENK expression between builders and control remained confined to the caudal mesopallium in the section 2.0 mm from median in the left hemisphere. The caudal medial mesopallium (CMM) at about 1 mm from median is part of the auditory forebrain pathway and stimulated by song perception (Jarvis *et al.* 2000). The region investigated here is located lateral to the CMM of Jarvis *et al.* (2000) and lateral, adjacent to the IMM as investigated in chicks by Horn (1998) who found the IMM to respond to auditory and visual stimuli. The IMM in chicks receives connections from most sensory systems and is also connected to descending paths of the telencephalon (Ball & Balthazart 2001). This area might be involved in the control of various aspects of behavioural activity including posture and locomotion (Ball & Balthazart 2001). Contrary to the above references who only report an increase in IEG expression as response to a stimulus, ZENK expression decreased after nest-building in the caudal mesopallium at 2.0 mm in the left hemisphere. This difference can be due to the different context investigated and the slightly different location compared to the above mentioned studies.

4.3.3 Nidopallium

In building birds, there were more stained cell nuclei in the caudal nidopallium from 3.0 mm to 4.0 mm in the right hemisphere than in controls. A decrease in ZENK expression after nest-building was observed in the intermediate nidopallium from 1.5 mm to 2.5 mm in the left hemisphere. Another region in the nidopallium showing an increase in ZENK expression after nest-building was the frontal nidopallium from 3.0 mm to 4.0 mm in the left hemisphere. In general, the nidopallium receives sensory input. Within it, three major areas with extratelencephalic input are known, namely field L (auditory input), entopallium (visual input), and nucleus basorostralis pallii (input from nervus trigeminus; Reiner *et al.* 2004). The entopallium, nucleus basorostralis pallii, and within field L, the area L2 – all three representing termination zones of thalamic projection – lack ZENK induction as a response to natural or artificial stimuli or administration of a GABAergic antagonist (Mello & Clayton 1994, 1995, Mello & Ribeiro 1998, Serries 2000, Mello 2002). Correspondingly, there was no ZENK expression in these three areas in the sections analysed in the present study. An increase in ZENK expression in building birds was observed in the caudal nidopallium from 3.0 mm to 4.0 mm from median in the right hemisphere. This region is located between the NCM and the caudolateral N (NCL), but could partially overlap with the NCL. The NCM as well as parts located dorsal and lateral to the caudal N, representing parts of the NCL, were also analysed, but no effect was found in these regions. The caudal N analysed here was in a songbird, whereas the studies on NCL were conducted on pigeons (Güntürkün 1997, Diekamp, Gagliardo, Güntürkün 2002). To confirm if this region is at least partially identical to the NCL, its function, anatomy, innervation, and properties of its neurons (Diekamp, Gagliardo, Güntürkün 2002) must be investigated. The NCL is regarded as functionally equivalent to the mammalian prefrontal cortex (Güntürkün 1997, Kalenscher *et al.* 2003), a structure involved in decision making and triggering of motor commands (Kim & Shadlen 1999, Tremblay & Schultz 1999). The NCL plays an important role in maintaining working memory for a variety of tasks (Diekamp, Gagliardo, Güntürkün 2002, Diekamp, Kalt, Güntürkün 2002) and consists of multiple subdivisions with regard to the distribution of neurotransmitters and neuropeptides in this area (Riters *et al.* 1999). Thus it is conceivable that this region is also involved in the control of nest-building behaviour.

4.3.4 Striatum

The striatum is regarded as part of the avian equivalent to the mammalian basal ganglia (Reiner *et al.* 1998, Farries & Perkel 2002, Reiner *et al.* 2004). Additionally, Fos expression was higher in the medial striatum during first courtship than in controls (Sadananda & Bischof 2002). The striatum is thought to be involved in the learning and maintenance of sequential motor actions that depend on sensorimotor integration (Aldridge & Berridge 1998), therefore both the medial and lateral striatum might well play a role in a weaverbird's nest-building behaviour. Of all analysed regions in the medial and lateral striatum, ZENK expression was significantly changed after nest-building in only one region. ZENK expression was downregulated after nest-building in the caudal medial striatum from 0.5 mm to 1.5 mm. This effect was only apparent in the left hemisphere, but the difference in ZENK expression between hemispheres in this region was smaller (negative) in building animals than in controls only for the section 0.5 mm from median, not in the region combined over the sections 0.5 mm, 1.0 mm, and 1.5 mm from median. No change in ZENK expression was found for the globus pallidus.

4.4 Hemispheric asymmetry of ZENK expression

A hemispheric asymmetry in IEG expression in birds was been previously observed by Lieshoff *et al.* (2004) in the tectum opticum with regard to ZENK expression and by Sadananda & Bischof (2004) in the hippocampus with regard to Fos expression. There is also evidence for hemispheric asymmetry with regard to enzyme activity and receptor characteristics, although these effects were apparent only 3 -13 hours after onset of training in imprinting (McCabe & Horn 1988, Sheu *et al.* 1993), and IEG expression did not differ in the investigated area between hemispheres (McCabe & Horn 1994). Evidence for a functional difference between hemispheres was also found by Nottebohm *et al.* (1976) for HVC and RA, but these effects were not apparent in IEG expression patterns (Jarvis & Nottebohm 1997). Most recent studies on IEG expression either investigated only one hemisphere or were looking at parasagittal sections in the one hemisphere and frontal sections in the other hemisphere, but not looking for differences between hemispheres. In this study, a difference was found in ZENK expression between hemispheres. The difference in ZENK expression between hemispheres increased with nest-building in the frontal hyperpallium apicale from 0.5 mm to 2.0 mm from median. This effect was caused by an increase after nest-building in the right hemisphere, but not in the left hemisphere. In all other regions for which ZENK expression was changed after nest-building in only one hemisphere, the differences between hemispheres did not change with nest-building. Either in only one hemisphere these effects were strong enough to be detected, and these regions are similarly linked to nest-building in both hemispheres, or an existing change in the difference in ZENK expression between hemispheres was not strong enough to be detected in other regions than the frontal hyperpallium apicale from 0.5 mm to 2.0 mm from median.

4.5 Semi-automated analysis of several regions

A new method was developed to automatically perform crucial parts of the analysis, allowing for screening of the 2x128 analysed forebrain regions in an objective manner and reasonable amount of time. This method can be developed further to analyse more and smaller frames, and to combine expression patterns of more than one IEG. The most important steps were the automated counting of stained cell nuclei in frames covering the whole forebrain, and the automated calculation of mean numbers of stained cell nuclei for all sampling frames lying within a defined region of interest. The first step, automatically counting stained cell nuclei in frames covering the whole forebrain, allows for a large number of distinct brain regions to be analysed. A limit in defining regions of interest is the size of the sample frames, which can also be adjusted to actual needs. The applied frame size of 300 x 300 µm is regarded as maximum size allowing an analysis of areas the size of HVC in Red Bishops. The only restriction for the reduction of the frame size is a minimal required number of cell nuclei contained within a frame. The latter step, automated calculation of mean numbers of stained cell nuclei for defined regions of interest, relied on a template created for each section individually. If neighbouring sections are to be stained for different IEGs, one template can be used to automatically determine expression of more than one IEG in regions of interest in these neighbouring sections, thus providing an economical method to analyse brain activity in fine detail and at large scale. Since the location of the counting frames is independent of the location of the regions of interest, regions of interest can be defined and modified after stained cell nuclei were counted for sampling frames. After frames are newly assigned to regions of interest, the automatic calculation of mean IEG expression for all regions of interest in a given section is only a simple mathematical procedure. The applied standardisation of the number of stained cell nuclei in all sampling frames for each individual separately eliminated differences in staining intensities between individuals. A possible negative effect could be the elimination of differences between experimental and control animals. Because sampling frames covered the whole forebrain of each analysed section, and for each individual the standardisation was based on all analysed frames of each individual, all regions and not only those involved in the control of nest-building were taken into consideration by the standardisation. The fact that there were significant differences in forebrain regions between experimental and control birds shows that this standardisation was not eliminating differences between experimental and control birds. The crucial step of course is the definition of regions of interest that are to be analysed. To allow for a more detailed template of defined regions of interest for each section, several different methods for staining of morphological features should be applied. This was not applied in the present study because its aim was to give a first overview of nest-building related ZENK expression in the Red Bishop's forebrain. Staining procedures for morphological features are e.g. cresyl violet, staining for acetylcholinesterase, and methionine enkephalin (mENK) staining (Durand *et al.* 1998). The method of automatic determination of brain activity for a number of defined regions is not restricted to IEGs, but can be applied to other procedures quantitatively determining brain activity. This method can be a fruitful contribution to the framework for analysing cerebral control of complex behaviours as outlined by Jarvis *et al.* (2002), involving molecular, anatomical, electrophysiological and behavioural levels of analysis. Chawla *et al.* (2004) published a method based on fluorescence in situ hybridization (FISH) of IEG expression for automated image analysis with the potential to be generalised to large-scale brain analysis that is both highly efficient and highly accurate. As outlined above, automated image analysis is only one step required for large-scale brain analysis. Another crucial step is the automated determination of brain activity in many different predefined regions of interest within the brain that enables the combination of several sources of information on brain activity.

4.6 Conclusion

In this pilot study on forebrain regions controlling nest-building behaviour in a weaverbird, six regions were found to change ZENK expression patterns in relation to nest building behaviour. As Jarvis & Mello (2000) stated, "… an avian forebrain pathway conveying specific sensory or motor information should contain nuclei from each of these major brain subdivisions", mesopallium, nidopallium, entopallium, arcopallium, and lateral and medial striatum. In this study all of these subdivisions contained regions with changes in ZENK expression related to nest-building. The results presented here should encourage future research in forebrain sensory and motor control of nest-building as a complex behavioural pattern, but at the same time treated with care because of the above mentioned considerations on the general experimental setup and statistics. The detection of ZENK expression is a widely used method for molecular mapping of brain activity, but only one of many tools to understand brain organisation (Jarvis *et al.* 2002). For a comprehensive analysis of regions involved in the sensorimotor control of nest-building, an approach following Jarvis *et al.* (2002) should be pursued, including other methods to map brain activity and to identify smaller, defined nuclei within major subdivisions of the forebrain.

5 References

Aldridge, J.W., Berridge, K.C. 1998. Coding of serial order by neostriatal neurons: a ‘‘natural action’’ approach to movement sequence. J Neurosci 18: 2777-2787

Ball, G.F., Balthazart, J. 2001. Ethological Concepts Revisited: Immediate Early Gene Induction in Response to Sexual Stimuli in Birds. Brain Behav Evol 57: 252-270

Ball, G.F., Tlemçali, O., Balthazart, J. 1997. Induction of ZENK protein after sexual interactions in male Japanese quail. Neuroreport 8: 2965-2970

Becker, R.A., Chambers, J.M., Wilks, A.R. 1988. The New S Language. Chapman & Hall, New York.

Bischof, H.-J. 2003. Neural mechanisms of sexual imprinting. Animal Biology 53(2): 89-112

Bolhuis, J.J., Cook, S., Horn, G. 2000. Getting better all the time: improving preference scores reflect increases in the strength of filial imprinting. Anim Behav 59: 1153-1159

Bolhuis, J.J., Zijlstra, G.G.O., den Boer-Visser, A.M., Van der Zee, E.A. 2000. Localized neuronal activation in the zebra finch brain is related to the strength of song learning. Proc Nat Acad Sci USA 97(5): 2282-2285

Brainhard, M.S., Doupe, A.J. 2002. What songbirds teach us about learning. Nature 417(6886): 351-358

Brenowitz, E.A. 1997. Comparative approaches to the avian song system. Journal of Neurobiology 33(5): 517-531

Catchpole, C.K., Slater, P.J.B,. 1995. Bird song. Biological themes and variations. Cambridge University Press, Cambridge

Chambers, J.M., Hastie, T.J. eds. 1992. Statistical Models in S. Chapman & Hall, New York

Chaudhuri, A. 1997. Neural activity mapping with inducible transcription factors. Neuroreport 8(13): III-VII

Chawla, M.K., Lin, G., Olson, K., Vazdarjanova, A., Burke, S.N., McNaughton, B.L., Worley, P.F., Guzowski, J.F., Roysam, B., Barnes, C.A. 2004. 3D-catFISH: a system for automated quantitative three-dimensional compartmental analysis of temporal gene transcription activity imaged by fluorescence in situ hybridization. J Neurosci Methods 139 (1): 13-24

Christy, B.A., Lau, L.F., Nathans, D. 1988. A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with ‘‘zinc finger’’ sequences. Proc Natl Acad Sci USA 85(21): 7857-7861

Clayton, D.F. 2000. The genomic action potential. Neurobiol Learn Mem 74(3): 185-216

Collias, E.C., Collias, N.E. 1964. The development of nest-building behaviour in a weaverbird. Auk 81: 42-52

Collias, E.C., Collias, N.E. 1973. Further studies on development of nest-bulding behaviour in a weaverbird (*Ploceus cucullatus*). Anim Behav 21: 371-382

Collias, N.E., Collias, E.C. 1984. Nest Building and bird behavior. Princeton University Press, Princeton, N.J.

Diekamp, B., Gagliardo, A., Güntürkün, O. 2002. Nonspatial and Subdivision-Specific Working Memory Deficits after Selective Lesions of the Avian Prefrontal Cortex. J Neurosci 22(21): 9573-9580

Diekamp, B., Kalt, T., Güntürkün, O. 2002. Working Memory Neurons in Pigeons. J Neurosci 22(4): RC210

Durand, S.E., Liang, W., Brauth, S.E. 1998. Methionine enkephalin immunoreactivity in the brain of the budgerigar (Melopsittacus undulatus): similarities and differences with respect to oscine songbirds. J Comp Neurol 393: 145-168

Eda-Fujiwara, H., Satoh, R., Bolhuis, J.J., Kimura, T. 2003. Neuronal activation in female budgerigars is localized and related to male song complexity. Eur J Neurosci 17: 149-154

Emlen, J.T.Jr. 1957. Display and mate selection in the whydahs and bishop birds. Ostrich 28: 202-214

Farries, M.A., Perkel, D.J. 2002. A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus. J Neurosci 22: 3776-3787

Güntürkün, O. 1997. Cognitive impairments after lesions of the neostriatum caudolaterale and its thalamic afferent in pigeons: functional similarities to the mammalian prefrontal system? J Hirnforsch 38 (1): 133-143

Hansell, M. 2000. Bird nests and construction behaviour. Cambridge University Press, Cambridge

Healy, S.D., Krebs, J.R. 1993. Development of hippocampal specialisation in a food-storing bird. Behav Brain Res 53: 127-131

Horn, G. 1998. Visual imprinting and the neural mechanisms of recognition memory. Trends in Neurosciences 21: 300-305

Jarvis, E.D., Mello, C.V. 2000. Molecular mapping of brain areas involved in parrot vocal communication. Comp Neurol 419(1): 1-31

Jarvis, E.D., Nottebohm, F. 1997. Motor-driven gene expression. Proc Natl Acad Sci USA 94: 4097-4102

Jarvis, E.D., Ribeiro, S., da Silva, M.S., Ventura, D., Vielliard, J., Mello, C.V. 2000. Behaviourally driven gene expression reveals song nuclei in hummingbird brain. Nature 406(6796): 628-632

Jarvis, E.D., Scharff, C., Grossman, M.R., Ramos, J.A., Nottebohm, F. 1998. For whom the bird sings: context-dependent gene expression. Neuron 21: 775-788

Jarvis, E.D., Smith, V.A., Wada, K., Rivas, M.V., McElroy, M., Smulders, T.V., Carninci, P., Hayashizaki, Y., Dietrich, F., Wu, X., McConnell, P., Yu, J., Wang, P.P., Hartemink, A.J., Lin, S. 2002. A framework for integrating the songbird brain. J Comp Physiol A 188: 961-980

Jarvis, E.D., Güntürkün, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W., Medina, L., Paxinos, G., Perkel, D.J., Shimizu, T., Striedter, G., Wild, J.M., Ball, G.F., Dugas-Ford, J., Durand, S.E., Hough, G.E., Husband, S., Kubikova, L., Lee, D.W., Mello, C.V., Powers, A., Siang, C., Smulders, T.V., Wada, K., White, S.A., Yamamoto, K., Yu, J., Reiner, A., Butler, A.B.; Avian Brain Nomenclature Consortium. 2005. Avian brains and a new understanding of vertebrate brain evolution. Nature Reviews Neuroscience 6(2): 151-159

Kalenscher, T., Diekamp, B., Güntürkün, O. 2003. Neural architecture of choice behaviour in a concurrent interval scheme. Eur J Neurosci 18: 2627-2637

Kim, J.N., Shadlen, M.N. 1999. Neural correlates of a decision in the dorsolateral prefrontal cortex of the macaque. Nature Neuroscience 2: 176-185

Kruse, A.A., Stripling, R., Clayton, D.F. 2000. Minimal experience required for immediate-early gene induction in zebra finch neostriatum. Neurobiol Learn Mem 74(3): 179-184

Lemaire, P., Revelant, O., Bravo, R., Charnay, P. 1988. Two mouse genes encoding potential transcription factors with identical DNA-binding domains are activated by growth factors in cultured cells. Proc Natl Acad Sci USA 85: 4691-4695

Lieshoff, C., Große-Ophoff, J., Bischof, H.-J. 2004. Sexual imprinting leads to lateralized and non-lateralized expression of the immediate early gene zenk in the zebra finch brain. Behav Brain Res 148(1-2): 145-155

Long, K.D., Salbaum, M. 1998. Evolutionary conservation of the Immediate Early Gene ZENK. Mol Biol Evol 15(3): 284-292

MacLean, G.L. Ed. 1993. Roberts' Birds of Southern Africa, 6th Edition, John Voelcker Bird Book Fund, Cape Town, South Africa

Maindonald, J., Braun, J. 2003. Data Analysis and Graphics Using R. Cambridge University Press, Cambridge

McCabe, B.J., Horn, G. 1988. Learning and memory: Regional changes in N-methyl-D-aspartate receptors in the chick brain after imprinting. Proc Natl Acad Sci USA 85: 2849-2853

McCabe, B.J., Horn, G. 1994. Learning related changes in Fos-like immunoreactivity in the forebrain after imprinting. Proc Natl Acad Sci USA 91: 11417-11421

Mello, C.V. 2002. Mapping vocal communication pathways in birds with inducible gene expression. J Comp Physiol A 188: 943-959

Mello, C.V., Clayton, D.F. 1994. Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. J Neurosci 14(11): 6652-6666

Mello, C.V., Clayton, D.F. 1995. Differential induction of the ZENK gene in the avian forebrain and song control circuit after metrazole-induced depolarization. J Neurobiol 26: 145-161

Mello, C.V., Nottebohm, F., Clayton, D.F. 1995. Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene’s response to that song in zebra finch telencephalon. J Neurosci 15(11): 6919-6925

Mello, C.V., Ribeiro, S. 1998. ZENK protein regulation by song in the brain of songbirds. J Comp Neur 393: 426-438

Mello, C.V., Vicario, D.S., Clayton, D.F. 1992. Song presentation induces gene expression in the songbird forebrain. Proc Natl Acad Sci USA 89: 6818-6822

Milbrandt, J. 1987. A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor. Science 238: 797-799

Nottebohm, F., Stokes, T.M., Leonard, C.M. 1976. Central control of song in the canary, *Serinus canarius*. J Comp Neurol 165: 457-486

Reiner, A., Medina, L., Veenman, L. 1998. Structural and functional evolution of the basal ganglia in vertebrates. Brain Res Brain Res Rev 28: 235-285

Reiner, A., Perkel, D.J., Bruce, L.L., Butler, A.B., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striedter, G., Wild, M., Ball, G.F., Durand, S., Gütürkün, O., Lee, D.W., Mello, C.V., Powers, A., White, S.A., Hough, G., Kubikova, L., Smulders, T.V., Wada, K., Dugas-Ford, J., Husband, S., Yamamoto, K., Yu, J., Siang, C., Jarvis, E.D. 2004. Revised Nomenclature for Avian Telencephalon and Some Related Brainstem Nuclei. J Comp Neurol 473: 377-414

Riters, L.V., Erichsen, J.T., Krebs, J.R., Bingman, V.P. 1999. Neurochemical evidence for at least two regional subdivisions within the homing pigeon (Columba livia) caudolateral neostriatum. J Comp Neurol 412: 469-487

Sadananda, M., Bischof, H.-J. 2002. Enhanced Fos Expression in the Zebra Finch (*Taeniopygia guttata*) Brain Following First Courtship. J Comp Neurol 448: 150-164

Sadananda, M., Bischof, H.-J. 2004. c-Fos Is Induced in the Hippocampus During Consolidation of Sexual Imprinting in the Zebra Finch (*Taeniopygia guttata*). Hippocampus 14: 19-27

Scheich, H., Zuschratter W. 1995. Mapping of stimulus features and meaning in gerbil auditory cortex with 2-deoxyglucose and c-Fos antibodies. Behav Brain Res 66: 195-205

Serries, E. 2000. Stimulusabhängige Expression des Immediate Early Genes ZENK im Vorderhirn des Staren (*Sturnus vulgaris*). Diploma thesis, Technical University of Munich

Shea, B.L. 1988. Algorithm AS 239, Chi-squared and Incomplete Gamma Integral, Applied Statistics. JRSS C 37: 466-473

Sheu, F.S., McCabe, B.J., Horn, G., Routtenberg, A. 1993. Learning selectively increases protein kinase C substrate phosphorylation in specific regions of the chick brain. Proc Natl Acad Sci USA 90: 2705-2709

Shin, C., McNamara, J., Morgan J., Curran, T., & Cohen, D. 1990. Induction of c-fos mRNA expression by after-discharge in the hippocampus of naive and kindled rats. Journal of Neurochemistry 55: 1050-1055

Skead, C.J. 1956. A study of the red bishop bird. Ostrich 27: 112-126

Smulders, T.V., DeVoogd, T.J. 2000. Expression of immediate early genes in the hippocampal formation of the black-capped chickadee (*Poecile atricapillus*) during a food-hoarding task. Behav Brain Res 114: 39-49

Sokal, R.R., Rohlf, F.J. 1995. Biometry 3rd edition. W.H. Freeman and Company, New York

Stripling, R., Volman, S.F., Clayton, D.F. 1997. Response Modulation in the Zebra Finch Neostriatum: Relationship to Nuclear Gene Regulation. J Neurosci 17(10): 3883-3893

Sukhatme, V.P., Cao, X.M., Chang, L.C., Tsai-Morris, C.H., Stamenkovitch, D., Verreira, P.C.P., Cohen, D.R., Edwards, S.A., Shows, T.B., Curran, T., LeBeau, M.M., Adamson, E.D. 1988. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. Cell 53: 37-43

Terpstra, N.J., Bolhuis, J.J., den Boer-Visser, A.M. 2004. An analysis of the neural representation of birdsong memory. J Neurosci 24 (21): 4971-4977

Tremblay, L., Schultz, W. 1999. Relative reward preference in primate orbitofrontal cortex. Nature 398: 704-708

Worley, P.F., Bhat, R.V., Baraban, J.M., Erickson, C.A., McNayghton, B.L., Barnes, C.A. 1993. Thresholds for synaptic activation of transcription factors in hippocampus—Correlation with long-term enhancement. J Neurosci 13: 4776-4786

Summary

In polygamous bird species, traits like song and plumage colouration can substantially influence individual reproductive success. Also important for reproductive success are nests because of their function to protect offspring. If nests or parts of them are built by one sex exclusively, nests can be chosen by the other sex. This is found in most weaverbird species. This thesis investigates nests and nest-building behaviour in Red Bishops (*Euplectes orix*). Red Bishops are a polygynous, colonially breeding weaverbird species widespread in sub-Saharan Africa. In this as in most weaverbird species, males alone build nest frames, which get accepted by females. Male nest-building behaviour was investigated over a course of three study seasons.

I investigated the effect of male nest-building behaviour and nest characteristics on reproductive success in the Red Bishop. Previous studies showed that male reproductive success is mainly determined by the total number of nests built which in turn depends both on the duration of territory tenure and nest-building performance (i.e. number of nests built per week). I could show that, apart from the number of nests built and territory tenure, the proportion of nests that became unusable within two weeks and the proportion of abandoned nest-building attempts also determined male reproductive success. The number of nests built by a male was related to territory tenure, the time needed to build a nest and the time delay between finishing a nest and starting with a new nest-building attempt. Male wing length, tarsus length and weight did not determine male reproductive success. Young males in their first breeding season were compared with older males to detect any age-related differences in reproductive effort and success. Young males had fewer nests accepted and built fewer nests than older males. In one season, young males held their territories for a shorter time than older males, and in another season, young males had longer delays between finishing a nest and starting a new nest-building attempt than older males. The observed differences in reproductive success between young and old males are best explained by a lack of experience and competence in young males. Nest measurements recorded in one study season revealed that nests with a higher breeding chamber density (number of fibres crossing a line of 1 cm length) and a larger entrance roof overlap had a higher probability of becoming accepted. A larger entrance roof overlap was related to increased durability of not

accepted nests, and a higher breeding chamber density was related to decreased probability of breeding failure.

Time budgets and nest-building efficiency were recorded for territorial male Red Bishops. An analysis of time budgets did not reveal any trade-offs between different behaviours in males. Instead, males differed in their general activity. The number of nests built by a male was related to the time spent with nest-building and nest-building efficiency in terms of the number of fibres woven in per minute spent with nest-building. Reproductive success was related to the total number of fibres woven in. There were no consistent patterns of the relationship between territory tenure, male-male interactions and the proportion of time spent in the territory while territorial.

Nests can be regarded as a commodity traded in a biological market. The Red Bishop’s breeding season as a biological market is characterised by considerable changes in demand for and supply of nests. Additionally, most nests became accepted while less than a week old, and young nests were more often accepted than expected by chance alone. In accordance with the preference for young nests, male nest-building activity in the whole colony correlated best with the number of nests newly accepted by females in the same and following week. Nests built at times of high breeding activity were built faster and with a shorter delay between two consecutive nests. In only one of three seasons, males with a more accurate adjustment of their building speed to breeding activity profited from higher reproductive success. The Red Bishop’s breeding system fulfils the requirements to be regarded as a biological market with strong temporal changes in demand and supply within a breeding season. Dynamics of the Red Bishop’s mating market with nests as traded commodities are discussed.

Nest-building is a behavioural pattern requiring complex sensory and motor control. In order to identify forebrain regions involved in the control of nest-building behaviour, forebrain activity during nest-building was assessed by the protein expression of the Immediate Early Gene (IEG) *zenk*. Until now it is not known what forebrain areas are involved in the integration of this behavioural pattern. For the analysis of ZENK expression in a number of regions of interest within the forebrain, a new method was developed that automated crucial steps in the analysis of forebrain activity based on IEG expression and thus facilitates the screening of brain activity in a large number of forebrain regions. By analysing ZENK protein expression as an

indicator for brain activity, a number of forebrain regions could be identified that showed changes in their activity in relation to nest-building behaviour. ZENK protein expression increased after nest-building in a region of the frontal hyperpallium apicale in the right hemisphere. In the caudal medial mesopallium a decrease in ZENK expression with nest-building was observed in the left hemisphere. In the nidopallium, three different regions showed changes in ZENK expression after nest-building. The first region, located in the frontal part of the left hemisphere showed an increase in ZENK expression. The second region was located in the left hemisphere intermediate in the frontocaudal axis, with a decrease in ZENK after nest-building. The third region was the caudal nidopallium in the right hemisphere with an increase in ZENK after nest-building. A region in the caudal Medial Striatum in the left hemisphere showed a decrease in ZENK expression after nest-building. These changes in ZENK expression were all observed in only one hemisphere. A test for differences between experimental and control animals in their differences between hemispheres was significant only for the frontal hyperpallium. The difference was larger in building animals than in controls, due to selective ZENK expression in the right hemisphere in building animals.

Zusammenfassung

In polygamen Vogelarten bestimmen oft Eigenschaften wie Gesang und Gefiederfärbung individuellen Fortpflanzungserfolg. Einen großen Einfluss auf Fortpflanzungserfolg üben auch Nester als Schutz der Nachkommen aus. Wenn Nester oder Teile von Nestern nur von einem Geschlecht gebaut werden, können Nester durch das andere Geschlecht ausgewählt werden. Dies ist bei vielen Webervogel-Arten der Fall. Die hier vorgestellte Dissertation untersucht Nester und Nestbauverhalten beim Oryxweber (*Euplectes orix*). Oryxweber sind eine polygyne, in Kolonien brütende Webervogelart, die in Afrika südlich der Sahara weit verbreitet ist. In dieser Studie wurde Nestbauverhalten der Männchen während drei Brutsaisons untersucht.

Ich untersuchte den Einfluss von Nestbauverhalten und von Eigenschaften der Nester auf Fortpflanzungserfolg in Oryxwebern. Frühere Arbeiten zeigten, dass Fortpflanzungserfolg der Männchen vor allem von der Gesamtanzahl pro Saison gebauter Nester abhängt, die wiederum sowohl von der Dauer, wie lange ein Territorium gehalten wurde und Nestbauleistung (Anzahl pro Woche gebauter Nester) abhing. Ich konnte zeigen, dass außer der Anzahl gebauter Nester und der Dauer, wie lange ein Territorium gehalten wurde, auch der Anteil Nester, die innerhalb von zwei Wochen nach Fertigstellung unbenutzbar wurden, und der Anteil aufgegebener Nest-Anfänge den Fortpflanzungserfolg eines Männchens beeinflussten. Die Anzahl gebauter Nester hing mit der Dauer, wie lange ein Territorium gehalten wurde, der zum Bau eines Nestes benötigten Zeit und der Pause zwischen dem Bau zweier aufeinander folgender Nester zusammen. Flügellänge, Tarsuslänge und Gewicht hatten keinen Einfluss auf Fortpflanzungserfolg der Männchen. Junge Männchen in ihrer ersten Brutsaison wurden mit älteren Männchen verglichen, um mögliche altersabhängige Unterschiede in der Nestbauleistung und im Fortpflanzungserfolg zu entdecken. Junge Männchen hatten weniger akzeptierte Nester und bauten weniger Nester als ältere Männchen. In einer Saison hielten junge Männchen ihr Territorium nicht so lange wie ältere Männchen, während in einer anderen Saison junge Männchen längere Pausen zwischen dem Bau zweier aufeinander folgender Nester hatten. Die beobachteten altersabhängigen Unterschiede im Fortpflanzungserfolg werden am besten durch mangelnde Erfahrung erklärt. In einer Saison wurden Nester

ausgemessen. Nester mit einer höheren Faserdichte in der Brutkammer und einem größeren Dach über dem Eingang wurden eher akzeptiert. Ein größeres Dach über dem Eingang erhöhte die Stabilität nicht akzeptierter Nester und eine höhere Faserdichte in der Brutkammer erhöhte die Wahrscheinlichkeit, dass ein Gelege flügge wurde.

Weiterhin wurden Zeitbudgets und Nestbaueffektivität territorialer Männchen untersucht. Eine Analyse der Zeitbudgets von männlichen Oryxwebern konnte keinen negativen Einfluss verschiedener Verhaltensmuster aufeinander feststellen. Stattdessen unterschieden sich Männchen in ihrer allgemeinen Aktivität. Des weiteren gab es einen Zusammenhang zwischen der Anzahl gebauter Nester mit der mit Nestbau verbrachten Zeit und der Nestbaueffizienz, gemessen in der Anzahl eingewebter Fasern pro Minute Nestbau. Fortpflanzungserfolg hing mit der Gesamtanzahl eingewebter Fasern zusammen. Es gab keine allgemeingültigen Zusammenhänge zwischen der Zeit, die ein Territorium gehalten wurde, Männchen-Männchen Interaktionen und dem Zeitanteil, der im Territorium verbracht wurde.

Nester können als ein Handelsgut in einem natürlichen Markt betrachtet werden. Die Brutsaison der Oryxweber als natürlicher Markt für Nester ist durch beträchtliche Schwankungen in Angebot und Nachfrage gekennzeichnet. Zusätzlich werden die meisten Nester akzeptiert, solange sie höchstens eine Woche alt sind und junge Nester wurden öfter akzeptiert als bei zufälliger Verteilung erwartet. Entsprechend der Bevorzugung junger Nester hängt Nestbauaktivität der Männchen am stärksten mit der Anzahl von Weibchen akzeptierter Nester in der gleichen und der folgenden Woche zusammen. Während hoher Brutaktivität wurden Nester schneller und schneller aufeinander folgend gebaut. In einer Saison steigerten Männchen ihren Fortpflanzungserfolg mit einer genaueren Anpassung der Baugeschwindigkeit an Brutaktivität. Das Brutsystem der Oryxweber erfüllt die Anforderungen, um als natürlicher Markt mit starken zeitlichen Schwankungen in Angebot und Nachfrage betrachtet zu werden. Die Dynamik dieses Marktes mit Nestern als Handelsgut wird diskutiert.

Nestbau ist ein Verhaltensmuster, das komplexe sensorische und motorische Steuerung erfordert. Um Vorderhirngebiete zu identifizieren, die an der Steuerung von Nestbauverhalten beteiligt sind, wurde Gehirnaktivität anhand der Protein-Expression des Immediate Early Gene (IEG) *zenk* beurteilt. Bis jetzt ist nicht bekannt, welche Vorderhirngebiete an der Verarbeitung dieses Verhaltensmusters beteiligt

sind. Für die Analyse der ZENK Expression in mehreren Gebieten des Vorderhirns wurde eine neue Methode entwickelt, die wichtige Schritte in der Bestimmung der ZENK Expression automatisierte und das Untersuchen einer großen Zahl von Gebieten im Vorderhirn ermöglichte. Durch die Bestimmung der Expression des Transkriptionsfaktors ZENK als Anzeiger für Gehirnaktivität wurden mehrere Vorderhirngebiete entdeckt, deren Aktivität sich durch Nestbauverhalten änderte. Im frontalen Hyperpallium apicale in der rechten Hemisphäre war ZENK Expression nach Nestbau höher. Im caudalen medialen Mesopallium in der linken Hemisphäre wurde nach Nestbau ein Rückgang der ZENK Expression beobachtet. Im Nidopallium zeigten drei verschiedene Regionen Änderungen in ZENK Expression nach Nestbau-Verhalten. Im frontalen Nidopallium in der linken Hemisphäre wurde ein Anstieg in der ZENK Expression nach Nestbau fetsgestellt. Im intermediären Nidopallium in der linken Hemisphäre fiel ZENK Expression nach Nestbau ab. Im caudalen Nidopallium in der rechten Hemisphäre stieg ZENK Expression nach Nestbau an. Im caudalen Medialen Striatum in der linken Hemisphäre ging ZENK Expression nach Nestbau zurück. Nur im frontalen Hyperpallium apicale war der Unterschied zwischen den Hemisphären bei bauenden Tieren größer als bei Kontrolltieren, was auf höhere Aktivität in der rechten Hemisphäre bauender Tiere zurückzuführen war.

Acknowledgements

First of all, I want to thank my supervisor Georg Klump. He provided the theme and all essential materials and equipment necessary for this thesis. He also supported me in securing funding for the thesis and the three field seasons. He is an inexhaustible source for suggestions, keeping me going until the very end of the thesis.

Thomas Friedl started the Red Bishop project in the Addo Park, and I was fortunate enough to become his successor. He introduced me to the Addo Park, not only to the study objects, but also to the people of the Park. In Germany, Thomas had always time when I wanted to discuss the intricacies of data analysis.

Franz Bairlain agreed to be my second supervisor on rather short notice and although he didn't have much time. I want to thank him for his engagement and inspiring discussions.

All people I shared the lab with over the time of this thesis I want to thank for the necessary chat and support, both in Munich and in Oldenburg. Ellen Serries introduced me to immunocytochemistry. Without her help I would have struggled much more than I did anyway, so a special thank goes to her. Malte Kittel proved to be an entertaining office mate and was responsible for the smiles of the day. Daniel Gehr made available helpful information about bird brain organisation. He also made sure we keep up the nice things of life like beach volleyball and cocktail evenings. Andrea Betz, Sonja Hofer, Ariane Santoso, Eva Wagner and the members of the lab of Prof. H.J. Leppelsack were good companions during the time in Garching.

Susanne Groß provided invaluable support in the lab and especially in taking care of the birds, in Munich as in Oldenburg. I was simply astonished by the good condition the birds acquired in her care and want to thank her very much.

Alice Aidnik, Garnet Bendig, Elli Groscurth, Peter Jaros, Peter Kanje, Karin Klink, Mirjam Knörrnshild, Kilian Köbrich, Ulrike Langemann, Julia Maier, Colette Pokar, Stephanie Schwettscher, Melanie Zokoll made life in the Oldenburg lab more aggreable. Mark Bee was hidden away in his office, but I found him anyway, and at the same time a fine lab mate. A big thank you goes to him for the time we spent together.

I want to thank Eric Jarvis for both the encouragement and the warning he gave me for my work with ZENK staining in a new behavioural context when I navigated through this part of my thesis.

Ralf Edler was mad enough to become not only my companion in the field but also another PhD student in the Red Bishop project. I enjoyed his company very much and miss the time together with him in Addo.

I want to thank the South African National Parks Board for the permission granted to continue the study on Red Bishops in the Addo Elephant National Park and to give me the opportunity to conduct research. The staff of the Addo Park supported me in every way I could ask for, especially the park wardens of my time, Fundisile Mketeni and Lucius Moolman. A special thanks for their cordial reception of the Germans goes to John and Melanie Adendorff.

My faithful if demanding guide in South Africa was Maghrieta Leyland who enabled me to appreciate the beauty of this country.

My former lab at the Free University of Berlin under Prof. Dietmar Todt provided excellent training from which I profited immensely in the course of this PhD thesis.

In the finishing and as with most PhD theses stressful phase, I received a lot of support from Steffi Hayen and Matthias Wehkamp and made it possible that I could fully concentrate on getting it all over and done.

A very special thanks goes to Louise Visagie for her never ending moral support and encouragement for this thesis, as well as her astounding ability to cheer me up in times of need. Above all, with her help I found a healthy balance between my PhD and all the other things in life that are not only nice but of vital necessity.

Without the help and support of my parents, many things in my life would not have been possible. One of these things is this thesis. I will think about a way to thank them properly.

Curriculum Vitae

Markus Metz

Persönliche Angaben

Postanschrift	c/o Wehkamp, Prinzessinweg 52A, 26122 Oldenburg
Email	Markus.Metz@uni-oldenburg.de
Tel.	privat +49 441 3006877
Geburtsdatum	29. September 1971
Geburtsort	Friedrichshafen
Staatsangehörigkeit	deutsch

Hochschulangehörigkeit

Promotion

Titel: Development and adaptive value of nest-building behaviour in the Red Bishop (*Euplectes orix*)

Betreuer: Prof. Georg M. Klump

Seit März 2002 an der Carl-von-Ossietzky Universität Oldenburg, finanziert durch die DFG

Von März 2000 bis März 2002 Doktorand an der Technischen Universität München, finanziert durch ein Landesstipendium der Technischen Universität München und des DAAD

Hauptstudium und Diplom in Biologie

April 1994 – April 1998, Freie Universität Berlin

Titel der Diplomarbeit: Antwortverhalten weiblicher Nachtigallen *(Luscinia megarhynchos)* auf das Vorspiel von Gesang

Betreuer: Prof. Dietmar Todt

Grundstudium und Vorprüfung Diplom Biologie

Oktober 1991 – Februar 1994, Universität zu Köln

Allgemeine Hochschulreife

1991, Graf-Zeppelin Gymnasium Friedrichshafen

Tagungen

Jahrestreffen der Deutschen Zoologischen Gesellschaft (DZG), Juni 2003, Berlin

Poster-Präsentation: Adjustment of male nest-building effort to female demand for new nests in the Red Bishop (*Euplectes orix*)

First European Conference on Behavioural Biology, August 2002, Münster

Poster-Präsentation: Nestbuilding performance and mating success in the polygynous red Bishop (*Euplectes orix*): the importance of building speed

ABS Meeting, Juni 1999, Bucknell, Pennsylvania, USA

Poster-Präsentation: Female choice for whistle songs in nightingales (*Luscinia megarhynchos*)

Freie Mitarbeit

April 1998 – März 1999

Teilnahme an einem Forschungsprojekt im Moremi Game Reserve, Okavango Delta, Botswana über akustische Kommunikation in frei lebenden Chacma-Pavianen (*Papio cynocephalus ursinus*). Betreuer: Prof. D.L. Cheney und R.M. Seyfarth, University of Pennsylvania, Philadelphia, USA.

Beschäftigung als Tutor in Lehrveranstaltungen

April 1995 – März 1998

Tutor am Zoologischen Institut der Freien Universität Berlin. Aufgaben: Vorbereiten und Halten von Vorlesungen, Betreuung in Praktika, Korrektur von Protokollen

Oktober 1993 – Februar 1994

Tutor am Institut für Biologie, Universität zu Köln: Begleitende Übungen zur Vorlesung Mathematik für Biologen

Oldenburg, den 16. März 2005

Oldenburg, den 17. Mai 2005

Erklärung gemäß § 10 Absatz (2) Punkt b) der Promotionsordnung der Fakultät für Mathematik und Naturwissenschaften der Carl von Ossietzky Universität Oldenburg vom 11.12.2003

Die vorgelegte Arbeit wurde selbständig verfasst und es wurden nur die angegebenen Hilfsmittel benutzt. Die Dissertation wurde bisher noch nicht in Teilen oder in Gänze veröffentlicht.

Markus Metz