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- Zebra finches in the Australian outback -



Social Influences during Adolescence on Adult Behaviour
in Zebra Finches (*Taeniopygia guttata*)

-

Underlying Mechanisms and Functional Consequences

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Summary

An individual's adult phenotype is not only influenced by its genetic predisposition, but also by environmental factors during ontogeny. This developmental plasticity enables an organism to produce a phenotype that is adapted to current or predicted future environmental conditions, enhancing the fitness of the individual at some point during its life. In many species, social influences during the prenatal and early postnatal period have long-lasting consequences for adult social behaviour. Lately, the adolescent life stage is being considered as another important developmental period of enhanced plasticity in which adult behaviour can be shaped. However, not much is known yet about the underlying mechanisms and fitness consequences of long-lasting behavioural modifications induced during adolescence. Based on research in mammals, it has been suggested that differences in social interactions modulate the secretion of sex steroid and glucocorticoid hormones, which in turn affect physiological maturation and the display of adult behaviour with consequences for fitness. In general, a more complex social environment has been proposed to improve adult social performance via increased social interactions.

The aim of this thesis was to investigate the mechanisms and consequences of variation in adult behavioural phenotypes induced by differences in the social environment during adolescence in a social avian species, the zebra finch (*Taeniopygia guttata*). Zebra finches are monogamous songbirds usually living and breeding in large colonies, but especially during the breeding season, the size of colonies can differ considerably. In addition, some pairs breed more solitarily at a distance from other pairs rather than in the colony. This might crucially affect social interactions of individuals during development, which in turn might influence maturational parameters and adult behavioural performance. An earlier study in which male zebra finches were kept in pairs or groups of juveniles during adolescence in the lab has shown that those males differ in the frequency of adult courtship and aggressive behaviour, which may represent adaptive behavioural modifications.

In the first study of this thesis, summarised in **chapter 2**, I investigated whether males reared in pairs or in groups of only juveniles, or in groups of juveniles and adults differ in the frequency of social interactions, endocrinological profiles (testosterone, corticosterone) and the development of adult song and adult plumage colouration during adolescence. Furthermore, I examined whether differences in developmental traits came along with differences in adult courtship and aggressive behaviour. I found that group-reared males had more social interactions and matured faster during adolescence than pair-reared males. In addition, group-reared males showed more courtship and aggression in adulthood, even though the strength of these effects differed between groups with and without adults. This suggests that effects of the adolescent social environment on adult behaviour in zebra

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finches are mediated via changes in social interactions and their effects on physiological processes underlying maturation. However, there was no evidence that the long-lasting differences in behaviour were related to changes in testosterone or corticosterone levels. Finally, the results further indicated that group-reared males differed from pair-reared males in their ability to adjust their behaviour to different types of interaction partners. They showed a high level of aggression towards socially less experienced and hence potentially inferior pair-reared opponents, but a low level of aggression towards equally skilled group-reared rivals. Pair-reared males did not show any adjustment of aggression to their opponents' characteristics. This might indicate a higher social competence of group-reared males, and is likely to affect fitness, for example by facilitating access to females when competing against inferior rivals.

In **chapter 3**, I further investigated the ability of males to adjust the expression of courtship and aggression as a function of available social information. Males were singly introduced into an unfamiliar flock of established breeding pairs and additional unpaired females. The mating status of conspecifics is especially important in a monogamous group-living species, such as the zebra finch, because the chance to secure a mate and the risk to get involved in costly fights depend on appropriate courtship and aggression shown towards potential mates and competitors. All males showed more courtship song towards unpaired females than towards paired females, and males from different rearing conditions did not differ in how much they preferred singing towards unpaired females. As found previously, pair-reared males showed overall less courtship singing than group-reared males. These results suggest that there is no difference in the ability to assess the suitability of females as potential mates between male zebra finches from the different social rearing environments. However, males appear to differ in how they compete with opponents. All males directed more aggression towards males than towards females, but group-reared males discriminated significantly more between male and female interaction partners than pair-reared males. These effects may be adaptive, because increased courtship singing and fighting skills of group-reared males might increase their attractiveness and competitiveness, and thereby influence their fitness in complex social settings.

In the study summarised in **chapter 4**, I focused on the consequences of variation in courtship and aggression of males from different social rearing environments during adolescence for reproductive success. A complex social context with high potential for females to be selective during mate choice and with high male competition was chosen, because differences in courtship and aggression, which potentially affect attractiveness and competitiveness, are most likely to have the strongest effect under such conditions. Males reared in juvenile pairs, juvenile groups and mixed-age groups during adolescence were introduced into an aviary with a limited number of females and were allowed to breed. I found

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that more group-reared males obtained paternity than pair-reared males. In addition, group-reared males sired a larger number of offspring in a larger number of nests and often attained multiple paternities. The increased reproductive success of group-reared males compared to pair-reared males indicates that zebra finch males reared in an enriched social environment during adolescence are adapted to a life under complex social conditions in adulthood. An increased attractiveness and competitiveness may enable group-reared individuals to accrue fitness gains in such an environment through higher mating success and increased extra-pair paternities.

In conclusion, my findings support the idea of adolescence as a sensitive period in which adult behaviour can be shaped by the social environment in zebra finches. Moreover, I present, for the first time, evidence that the effects of the adolescent social environment on adult behaviour may be mediated via changes in social interactions, affecting physiological maturation of individuals. A higher frequency of interactions in an enriched social environment seems to accelerate maturation of zebra finch males and to result in behavioural modifications that are beneficial under complex social conditions with high competition in later life. Hence, this thesis provides the first evidence for adaptive phenotypic shaping by the social environment during adolescence in zebra finches.

Chapter 1

General Introduction

S. Bölting

Phenotypic plasticity

Individual variation in physiology, morphology and behaviour is common in a wide range of animal species, including humans. To a certain extent, this variation can be traced back to differences in genetic predisposition. However, when exposed to different environmental conditions, even genetically identical individuals may display quite distinct characteristics (Archer et al. 2003, Freund et al. 2013). The ability of a single genotype to produce various alternative forms of physiological, morphological and behavioural traits in response to different environmental conditions is typically referred to as “phenotypic plasticity” (West-Eberhard 1989). The set of phenotypes that can emerge from a given genotype, however, is not unlimited, but confined by an individual’s “reaction norm” (Via et al. 1995). Latest studies in Amazon mollies (*Poecilia formosa*) indicate that phenotypic shaping might not only be guided by an interaction of genetic information and environmental cues, but also by differences in microenvironments or epigenetic variation (Bierbach et al. 2017). However, in the following phenotypic plasticity will be used according to the current research paradigm.

Originally, the term “phenotypic plasticity” covered all types of phenotypic variation induced by the environment (Stearns 1989). More recently, often different terms are used to refer to different major categories of phenotypic plasticity, i.e. irreversible or reversible phenotypic modifications (Table 1). According to Piersma & Drent (2003), “developmental plasticity” refers to irreversible inter-individual variation in traits resulting from environmental differences during development. Many life-history traits belong to this category. For example, in female (Trillmich et al. 2009) and male (Guenther et al. 2014) wild cavies (*Cavia aperea*), the age at maturity is strongly affected by photoperiodic conditions individuals are born into. In contrast to developmental plasticity, “phenotypic flexibility” refers to reversible intra-individual variation of traits that can be altered more than once in response to current and more rapid environmental changes (Piersma & Drent 2003). A typical morphological trait that can be flexibly adjusted is body size. For example, marine iguanas (*Amblyrhynchus cristatus*) (Wikelski & Thom 2000) and sea urchins (*Diadema antillarum*) (Levitan 1989) alter their body size according to food abundance and, in case of sea urchins, to social density (Levitan 1989). Behaviour can also be flexibly adjusted to different contexts. In a laboratory strain of house mice (*Mus musculus*), individuals show aggressive behaviour towards conspecifics when assigned the dominant position in social hierarchy contests, but submission when assigned the subordinate position (Branchi et al. 2006). Notably, mice that have grown up in the enriched social environment of a communal nest exhibit an increased adjustment of their behavioural response to the social context than individuals that were reared by only a single mother (Branchi et al. 2006). Similarly, in a cooperatively breeding cichlid (*Neolamprologus pulcher*), a more appropriate use and adjustment of aggression and submission has been detected in individuals that have grown up with peers and adults compared to individuals

grown up with peers only (Arnold & Taborsky 2010). Thus, the degree of phenotypic flexibility shown by organisms may still be affected by environmental cues during development.

The above mentioned examples illustrate that phenotypic variation can be caused by various abiotic and biotic factors. Indeed, also variation in temperature (Bull 1980, Biro et al. 2010) or the presence of predators (Krueger & Dodson 1981, Lively 1986) affects the phenotypic development of individuals. However, in this thesis, I will primarily focus on the effects the social environment (presence of conspecifics) exerts on an individual's phenotype.

Table 1: Characteristics of two major categories^a of phenotypic plasticity^b.

Category of	Phenotypic change	Variability within
phenotypic plasticity	is reversible	a single individual
Developmental plasticity	No	No
Phenotypic flexibility	Yes	Yes

^a modified after Piersma & Drent (2003)

^b Phenotypic plasticity is referred to as the ability of a single genotype to produce various alternative forms of physiological, morphological and behavioural traits in response to different environmental conditions (West-Eberhard 1989)

Sensitive windows for adaptive phenotypic shaping

There are two main functional explanations for the occurrence of phenotypic variation induced by environmental cues during development. On the one hand, extremely unfavourable conditions during ontogeny may disrupt important developmental processes and maladaptive phenotypic modifications may occur. For example, zebra finches raised in colonies without adult males exhibit a reduced preference for opposite-sex partners in adulthood and are less likely to successfully pair with them compared to individuals that were raised in colonies with adult males (Adkins-Regan & Krakauer 2000). The impaired ability to choose the opposite sex as a partner, which is a critical component of mate choice, is likely to significantly reduce reproductive success in this pair-bonding species (Adkins-Regan & Krakauer 2000). On the other hand, environmental cues during development may induce phenotypic modifications that are adapted to the demands of an individual's environment. Hence, such phenotypic modifications should improve the fitness of the individual, either

directly or at a later time point in life when environmental conditions predicted earlier come true. This has been proposed by the “Predictive Adaptive Response” hypothesis (Bateson et al. 2014) and the “Environmental Matching” hypothesis (Monaghan 2008). A lot of research about early environmental effects supports or strongly indicates an adaptive value of phenotypic modifications. For example, in meadow voles maternal photoperiodic history during pregnancy affects the extent of fur development and hence the winter preparedness of pups (Lee & Zucker 1988). In a variety of mammal species (mice: Allen & Haggett 1977, Harvey & Chevins 1984, Crump & Chevins 1989; rats: Dahlöf et al. 1977; guinea pigs: Kaiser & Sachser 2001) high social density conditions during pregnancy of dams lead to a reduction, demasculinization or feminization of the male offspring’s sexual behaviour. It has been suggested that such behavioural modifications may prevent young males from being attacked by older and more experienced males in a large population and hence increase fitness (Kaiser & Sachser 2001).

In many species there seem to be certain life periods or stages, in which adaptive phenotypic shaping is especially likely to occur. These periods or stages, considered as “sensitive windows”, are characterized by (i) an increased frequency of cues, (ii) an increased informative value of cues, (iii) increased fitness benefits of information and / or (iv) reduced constraints on plasticity (Fawcett & Frankenhuis 2015). More precisely, this means first that trait expression should be plastic whenever there might be increased information on how environmental conditions look like or will look like in the future and whenever information about present or future environmental conditions is reliable and reduces the degree of uncertainty about these conditions. Furthermore, trait expression should be plastic whenever information is valuable and whenever responding to information with specific phenotypic adjustments is not limited or costly (Fawcett & Frankenhuis 2015). It is furthermore thought that as individuals get better informed and less uncertain about environmental conditions, they should be less influenced by new cues (Fawcett & Frankenhuis 2015). This might explain why in stable environments in which future conditions can be reliably predicted, the occurrence of phenotypic modifications declines with age. Indeed, the prenatal and the early postnatal period, in which individuals are most naïve about their surrounding environment, are the most commonly recognized sensitive windows that have been extensively investigated (e.g. Adkins-Regan & Krakauer 2000, Kaiser & Sachser 2005, Branchi 2009) since the pioneering work on early environmental influences conducted by ethologists such as Lorenz (Lorenz 1935) and Immelmann (Immelmann 1969, 1972). However, in variable environments, the reliability of cues is highest close to the situation that is indicated by the cues. Therefore, when environmental input is variable, individuals should retain a high degree of plasticity beyond the very early life stages or even throughout their whole life, such as found in the cichlid fish *Astatotilapia burtoni* (Maruska & Fernald 2013).

A life stage in which many individuals experience considerable change in the social environment is adolescence. However, environmental effects during adolescence on phenotypic traits have only recently received increased attention. Adolescence is broadly defined as the developmental transition from childhood to adulthood, including the attainment of sexual maturity and changes in associated neural circuits, endocrine systems, morphology and behaviour (Spear 2000, Sisk & Foster 2004, Sisk & Zehr 2005, Sachser et al. 2011). In addition, social maturity is also attained during adolescence (Sisk & Zehr 2005). Through emigration from their natal group (Baker 1978) and shifting from primarily interacting with parents to primarily interacting with peers and other adults (Zann 1996, Spear 2000), young gain knowledge about social rules and learn how to appropriately behave towards conspecifics (Sachser et al. 2011). As sexual maturity approaches, social cues regarding sexual traits, such as the intensity of mate competition individuals will be exposed to, might be especially important during adolescence. Indeed, in several animal species, such as hamsters (Ferris et al. 2005), guinea pigs (Sachser et al. 2011, 2013, Zimmermann et al. 2017) and zebra finches (Mariette et al. 2013, Ruploh et al. 2013, Honarmand et al. 2015), the adolescent social environment crucially affects sexual behavioural traits. However, detailed knowledge about the underlying mechanisms and the consequences of behavioural modifications induced by variation in the social environment during adolescence is scarce.

Underlying mechanisms of phenotypic variation

Especially for social species, interactions with conspecifics may be the most important source providing information on environmental conditions, for example on population density (Taborsky 2016). The higher the density of a population is in which an individual lives, the higher is typically the social stimulation it receives. Social interactions furthermore provide the opportunity to acquire behaviour patterns that are needed for life with conspecifics. In doing so, an increased frequency of interactions with a larger variety of interaction partners, as found in more complex social settings, increases social performance (Sachser 1998, Bastian et al. 2003, Branchi et al. 2006, Sachser et al. 2011, Arnold & Taborsky 2010). Therefore, it seems likely that the frequency or type of social interactions individuals experience crucially influence the development of phenotypic traits. However, information conveyed via social interactions may not always directly be acquired by an individual, but also indirectly and prenatally via the social interactions experienced by its parents, in most cases its mother. Increased social stimulation typically leads to alterations in the concentration of different hormones, for example androgens or glucocorticoids (Christian 1960, Sachser & Lick 1989, Wingfield et al. 1990), which can be prenatally transmitted from a mother to her young. In mammals, hormone transmission from a mother to her young occurs via the bloodstream (Sachser et al. 2013), while in birds transmission occurs via

deposition of hormones in the egg yolk (Schwabl 1997, von Engelhardt & Groothuis 2011). It has therefore been suggested that via this prenatal transmission of hormones, mothers affect the development of the endocrine system, brain and behaviour of embryos, thereby preparing their offspring to the environmental conditions that young are likely to encounter after birth or hatching. For example, black-headed gull (*Larus ridibundus*) mothers deposit varying levels of steroid hormones in their eggs according to laying order and breeding location, thereby adjusting the growth and competitiveness of their offspring to hatching asynchrony and population density (Groothuis & Schwabl 2002). Once born or hatched individuals acquire information about their environment via direct interactions with conspecifics. For example, in the early postnatal phase, the amount of maternal care behaviour provided to young, which can reflect the environmental adversity experienced by the mother, determines the neuroendocrine stress responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis and behavioural parameters in the offspring of domesticated rats (*Rattus norvegicus*) (Meaney 2001). Also in the adolescent life stage, the importance of social interactions for triggering neuroendocrine and thereby behavioural changes has been demonstrated. Compared to male guinea pigs (*Cavia aperea f. porcellus*) reared in mixed-sex pairs during adolescence, males reared in mixed-sex colonies experience an increased frequency of social interactions, resulting in increased levels of testosterone in the blood and a reduced HPA responsiveness to stress (Lürzel et al. 2010, Sachser et al. 2011). As a consequence, colony-reared males exhibit low levels of aggression towards same-sex opponents and integrate into an unknown group of conspecifics without physiological disadvantages (Sachser et al. 2011). In contrast, pair-reared males display high levels of aggression and suffer from severe endocrine stress responses when introduced into an unfamiliar group, but gain increased reproductive success under conditions of low population density compared to colony-reared males (Sachser et al. 2011, Zimmermann et al. 2017). Hence, male guinea pigs reared in environments of differing social complexity during adolescence seem to be phenotypically adapted to a life under similar conditions in adulthood. It has been proposed that colony-reared males follow a “queuing strategy” in order to prevent harmful interactions with older males in the colony until they are physically strong enough to fight against them for access to females. Pair-reared males, in contrast, seem to display a “resource defence strategy”, which is rather adaptive under environmental conditions of low population density (Sachser et al. 2011, 2013). Similar to these findings in guinea pigs, male zebra finches (*Taeniopygia guttata*) reared in groups during adolescence have been found to be less aggressive and to show less courtship in adulthood than males reared in pairs (Ruploh et al. 2013). Furthermore, group-reared males integrated better into an unknown group of conspecifics (Ruploh et al. 2014a). However, the underlying hormonal

mechanisms and the potential fitness consequences of differences in courtship and aggression induced during adolescence have not yet been investigated in zebra finches.

Notably, research about fitness consequences of behavioural alterations should take into account not only the total frequency of a behavioural pattern, but also the context during which it is expressed and towards which individuals it is directed. When introduced into an unfamiliar environment, male brown-headed cowbirds (*Molothrus ater*) previously kept in dynamic flocks and males kept in stable flocks did not differ in their total frequency of aggression and courtship. However, dynamic-flock males directed aggression and courtship more selectively towards different interaction partners than stable-flock males, resulting in increased mating success (White et al. 2010, Gersick et al. 2012). A selective use of behaviour towards different interaction partners might indicate high behavioural flexibility. Behavioural flexibility is an important factor underlying social competence, which is the ability of an individual to optimise its social behaviour depending on available social information (Taborsky & Oliveira 2012). Hence, increased behavioural flexibility is likely to contribute to an individual's fitness.

In order to understand how the effects of the adolescent social environment on adult courtship and aggressive behaviour of zebra finch males are mediated, it is essential to investigate social interactions and hormonal profiles of individuals reared in different social environments during adolescence. The adaptive significance of phenotypic modifications can be confirmed by investigating the fitness consequences of differences in behaviour under different environmental conditions in adulthood.

Study species – the zebra finch (*Taeniopygia guttata*)

Habitat and social system

The experiments presented in this thesis were conducted using the zebra finch (*Taeniopygia guttata*) as a study species. Zebra finches are small Australian passerines from the family of Estrildid finches (Estrildidae) that are distributed over almost the whole Australian continent, except some coastal areas and the island of Tasmania (Fig. 1). They favour dry open grasslands with wooded areas close to waterholes, where they usually live in flocks of up to several hundred individuals (Zann 1996). However, in zebra finches, social density can vary considerably, especially during the breeding season (Zann 1996). In addition, some pairs separate from the colony when breeding and rear their offspring up to 800 m away (Zann 1996, Mariette & Griffith 2012, 2013). Chicks are altricial and spent about the first 20 days in their nest, during which interactions only occur with their parents and siblings. At around day 20 post-hatching chicks fledge, but fledglings still spend most of their time nearby their natal nest and may only interact with other individuals in the same bush (Zann 1996). Only at

around day 30 post-hatching offspring start to follow their parents to the main colony, where they encounter other conspecifics and increasingly interact with peers. At this time, offspring also start to forage on their own and become fully nutritionally independent from their parents only a little later, around day 35–40 post-hatching (Zann 1996). Given the natural variation in breeding density, the frequency and type of social interactions experienced by offspring during early development varies considerably, even within a single population.



Fig. 1: Map of the Australian continent. The distribution area of zebra finches is highlighted in green (del Hoyo J, Elliott A, Christie DA. eds. (2010). Handbook of the Birds of the World. Vol 15. Weavers to New World Warblers. Lynx Edicions, Barcelona).

Morphology and behaviour

Adult zebra finches reach a body length of 10–11 cm and a weight of 10–17.5g in the wild (Zann 1996), but domesticated birds are typically heavier. Adults exhibit sexual dimorphism in plumage colouration and behaviour. Females are light grey with a white belly, an orange beak and two black stripes in their face (Fig. 2A). In addition to this, males exhibit black stripes on their throat, a black bar on their breast, chestnut coloured feathers with white spots along their flanks and bright orange cheek feathers (Fig. 2A) (Zann 1996). Both females and males use different vocal sounds to communicate, but only males sing (Zann 1996). Singing can be undirected or used during courtship and can be easily quantified, as males repeat a

series of relatively rigid motifs in a stereotyped manner (Zann 1996). Even though zebra finches are highly social and gregarious, they do display aggression, for example to defend food, nest material, a breeding spot or their mate (Zann 1996). Chasing is the most intense form of aggression that is shown by zebra finch males and females and can be easily quantified, too. Juvenile zebra finches of both sexes are grey and have a black beak (Fig. 2B). They start to develop the adult plumage and beak colouration at around day 35–40 post-hatching. Sexual dimorphism in behaviour may even occur as early as 25–30 days after hatching. Under natural social conditions, both morphological and behavioural traits are fixed and the majority of individuals start to reproduce at an age of 100-110 days (Zann 1996).

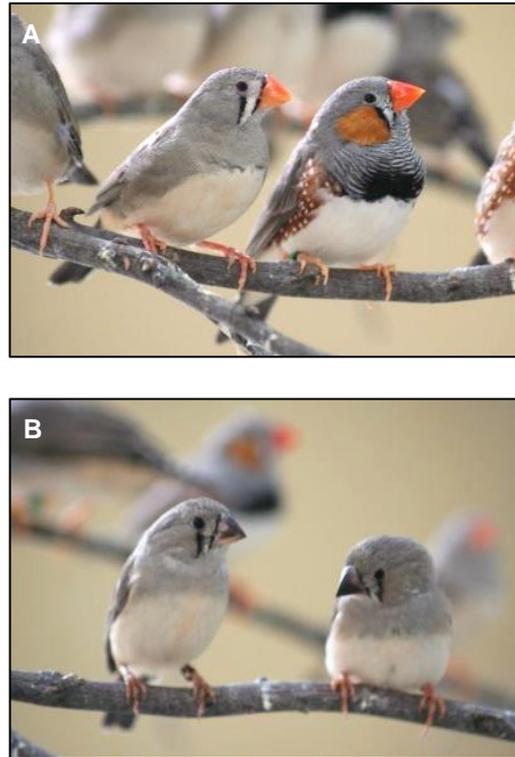


Fig. 2: Zebra finch morphology. **A)** Adults. Left: female, right: male. **B)** Juveniles. Age: about 40 days.

Effects of the social environment during development

Zebra finch development can be easily studied under laboratory conditions, as individuals successfully mate, reproduce and rear their young under a variety of artificial social housing conditions (Griffith et al. 2017). Many studies conducted in the lab revealed that the social environment experienced during ontogeny significantly influences the morphological and behavioural development of zebra finch young. For example, males kept in social isolation during early development exhibit a significant delay in sex-specific plumage colouration (Leader & Nottebohm 2006) compared to socially reared controls. In addition, rearing in social deprivation results in a disrupted development of adult song (Morrison & Nottebohm 1993, Jones et al. 1996). There is some evidence that prenatally or in the early postnatal life stage, phenotypic modifications are induced by endocrine changes (Groothuis et al. 2005, Spencer et al. 2003) in zebra finches. It has furthermore been found that during early development, changes in testosterone occur in socially housed individuals (Pröve 1983, Adkins-Regan et al. 1990) and that the early social environment has long-lasting effects on the endocrine stress response (Banerjee et al. 2012). Hence, endocrine changes during early development may depend on social stimulation. However, the mechanisms underlying phenotypic modifications induced during the life stage of adolescence as well as the consequences of such modifications for fitness have not yet been investigated. Given that

the above-mentioned behavioural and morphological changes of young zebra finches between day 30 and 110 post-hatching closely resemble those undergone by individuals during the life stage of adolescence in other species, this time period has been considered to represent adolescence in zebra finches (Adkins-Regan & Leung 2006, Ruploh et al. 2013). Experiments aiming to investigate how effects of the social environment during adolescence are mediated on zebra finch adult behaviour should therefore focus on this time window.

Aims and outline of the thesis

In this thesis I investigate the mechanisms and consequences of behavioural modifications induced by the social environment during adolescence in the zebra finch (*Taeniopygia guttata*). As outlined above, it has been suggested in other species that effects of the social environment during adolescence on adult behaviour are adaptive and mediated via differences in social interactions affecting sex steroid as well as glucocorticoid levels and maturation (Sachser et al. 2011, 2013, Zimmermann et al. 2017). Therefore, in the following three chapters, experiments on effects of differing adolescent social experience on social interactions and physiological maturation during development and on the frequency and use of adult courtship and aggressive behaviour in different contexts are described. In addition, consequences of adult behavioural differences for reproductive success are investigated. In the final chapter, the main findings of the experiments are summarised and discussed and ideas for the direction of future research are proposed.

In **chapter 2**, experiments focused on the behavioural, endocrinological and morphological development of individuals kept in different social environments during adolescence and on the question whether differences in developmental traits are associated with differences in adult courtship and aggressive behaviour. Male zebra finches were kept in mixed-sex juvenile pairs, in mixed-sex juvenile groups or in mixed-sex mixed-age groups to compare the effects of differing levels of social complexity. Individuals housed under more complex conditions were expected to have more social interactions, to exhibit increased or earlier peaks of testosterone as well as corticosterone and to exhibit reduced adult courtship and aggression as a strategy to prevent costly competition with older and more experienced rivals in their environment.

The experiments presented in **chapter 3** investigated whether males differed in the adjustment of courtship and aggression according to the mating status of their interaction partners, which should be highly relevant for fitness. Experimental males from juvenile pairs, juvenile groups and mixed-age groups were introduced into a flock composed of two established breeding pairs and two additional females without a mate. It was expected that males reared in groups would use courtship towards females and aggression towards males more appropriately than males reared in pairs, due to their increased social experience with

male and female conspecifics during adolescence. It was also expected that the behavioural performance of males reared with and without adults would differ due to differences in learning opportunities during development.

Last, the adaptive value of the males' courtship and aggressive behaviour to the conditions of their social rearing environment was experimentally investigated. Therefore, in **chapter 4**, it was tested whether male reproductive success in adulthood differed depending on the complexity of a social setting. It was predicted that males reared in groups would, compared to males reared in pairs, obtain higher reproductive success in a social setting with increased potential for competition, due to a more appropriate behavioural performance towards opponents and potential mates.

In **chapter 5**, the findings of the thesis regarding the mechanisms and consequence of behavioural modifications induced by the social environment during adolescence in zebra finches are summarised and discussed in a broader context. Furthermore, I present an outlook of promising avenues for future research that could help to disentangle the relationship between the social environment, social interactions, endocrine changes and adult behaviour, and allow to evaluate the adaptive value of behavioural modifications in zebra finches.

Chapter 2

Effects of the social environment during adolescence on the development of social behaviour, hormones and morphology in male zebra finches (*Taeniopygia guttata*)

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Abstract

Background Individual differences in behaviour are widespread in the animal kingdom and often influenced by the size or composition of the social group during early development. In many vertebrates the effects of social interactions early in life on adult behaviour are mediated by changes in maturation and physiology. Specifically, increases in androgens and glucocorticoids in response to social stimulation seem to play a prominent role in shaping behaviour during development. In addition to the prenatal and early postnatal phase, adolescence has more recently been identified as an important period during which adult behaviour and physiology are shaped by the social environment, which so far has been studied mostly in mammals. We raised zebra finches (*Taeniopygia guttata*) under three environmental conditions differing in social complexity during adolescence - juvenile pairs, juvenile groups, and mixed-age groups - and studied males' behavioural, endocrine, and morphological maturation, and later their adult behaviour.

Results As expected, group-housed males exhibited higher frequencies of social interactions. Group housing also enhanced song during adolescence, plumage development, and the frequency and intensity of adult courtship and aggression. Some traits, however, were affected more in juvenile groups and others in mixed-age groups. Furthermore, a testosterone peak during late adolescence was suppressed in groups with adults. In contrast, corticosterone concentrations did not differ between rearing environments. Unexpectedly, adult courtship in a test situation was lowest in pair-reared males and aggression depended upon the treatment of the opponent with highest rates shown by group-reared males towards pair-reared males. This contrasts with previous findings, possibly due to differences in photoperiod and the acoustic environment.

Conclusion Our results support the idea that effects of the adolescent social environment on adult behaviour in vertebrates are mediated by changes in social interactions affecting behavioural and morphological maturation. We found no evidence that long-lasting differences in behaviour reflect testosterone or corticosterone levels during adolescence, although differences between juvenile and mixed-age groups suggest that testosterone and song behaviour during late adolescence may be associated.

Background

In many species, the social environment during ontogeny (White et al. 2002, Sachser et al. 2011, Mariette et al. 2013) influences the development of adult social behaviour (Adkins-Regan & Krakauer 2000, Delville et al. 2003, Sundström et al. 2003, Field & Waite 2004, Arnold & Taborsky 2010, Siegeler et al. 2011). However, the importance of social experiences for adult behaviour has mostly been demonstrated for the prenatal and early postnatal phase (e.g. Lorenz 1935, Immelmann & Suomi 1981, Branchi 2009), and only more recently has evidence accumulated that the social environment during adolescence is also crucial (Curley et al. 2011, Sachser et al. 2011, 2013). Adolescence can be defined as the gradual transition from childhood to adulthood (Spear 2000) and is characterised by marked neuronal, endocrine, morphological and behavioural changes (Spear 2000, Sisk & Zehr 2005, Blakemore 2008, Buwalda et al. 2011, Brown & Spencer 2013). Increases in sex steroids produced by the hypothalamo-pituitary-gonadal (HPG) axis during adolescence are critical in the development and regulation of the reproductive system, adult morphology, reproductive behaviour and sexual maturity (Nottelmann et al. 1987, Sisk & Foster 2004, Sisk & Zehr 2005, Adkins-Regan & Leung 2006, Schulz et al. 2009). In addition, increases in glucocorticoid hormones produced by the hypothalamo-pituitary-adrenal (HPA) axis in response to new stimuli and stressors (Sachser et al. 2011, Brown & Spencer 2013), but see (Hennessy et al. 2006), may be important when new environments or unfamiliar conspecifics are encountered during adolescence. Importantly, sex steroid and glucocorticoid levels are affected by social experiences (Harvey et al. 1984, Wingfield et al. 1990, Silverin 1998, Goymann et al. 2007). This suggests that variation in social experiences during adolescence may have long-lasting behavioural and morphological consequences via organisational effects of these hormones.

During adolescence, the social environment of juveniles changes considerably in many species as they gain independence from their parents and increasingly interact with other adults and peers (Zann 1996, Spear 2000). Although these interactions may be stressful, as indicated by increased glucocorticoid levels (Lürzel et al. 2010, Sachser et al. 2011), they allow juveniles to practice important behaviour which they will need in adulthood to reproduce successfully, such as courtship and aggression (Meaney & Stewart 1981, Kaiser & Sachser 2001, White et al. 2002). The size, age and sex composition of groups affect the amount and type of social interactions juveniles experience, and these are thought to affect future behaviour through learning and neuroendocrine changes (Bandura 1977, Galef et al. 2005, Choleris et al. 2009). Variation in social interactions during adolescence may thus adaptively adjust reproductive behaviour to the social conditions that juveniles are likely to encounter as adults. Studies in a variety of species, e.g. brown-headed cowbirds (*Molothrus ater*), (West & King 1988, White et al. 2002, Gersick et al. 2012), daffodil cichlids

(*Neolamprologus pulcher*) (Arnold & Taborsky 2010), guinea pigs (*Cavia aperea f. porcellus*) (Sachser et al. 2011, 2013) and zebra finches (*Taeniopygia guttata*) (Mariette et al. 2013, Ruploh et al. 2014a) suggest that a more complex early social environment improves adult social competence.

The most comprehensive studies on the effects of the social environment during adolescence on social interactions, hormones and adult behaviour have been conducted in guinea pigs (*Cavia aperea f. porcellus*) (Lürzel et al. 2010, 2011a, 2011b, Sachser et al. 2011, Hennessy et al. 2015). Guinea pigs are polygynous rodents, in which dominant males aggressively monopolise and court all available females at low social densities, whereas at high social densities males tolerate each other and do not court females bonded to another male. The studies found that males growing up in a mixed-sex group with adults had significantly more social interactions and higher testosterone (T) and cortisol concentrations during adolescence than males growing up in a mixed-sex pair with a female peer. As adults, group-reared males, compared to pair-reared males, showed a lower cortisol response and less aggressive behaviour in an encounter with an unfamiliar male in a new environment and less courtship behaviour towards unfamiliar females (Sachser et al. 1994, Sachser et al. 2011). The researchers suggested this reflects an adaptive “queuing strategy” (Sachser et al. 2011). The authors proposed the following mechanisms by which the adolescent social environment causes adult behavioural modifications: A high frequency of social interactions during adolescence increases T concentrations which reduces the adult cortisol responsiveness that controls the display of adult aggressive behaviour via organisational effects (Sachser et al. 2011).

Interestingly, in zebra finches, the social environment during adolescence has very similar effects on adult courtship and aggression as in guinea pigs. Zebra finch males housed in mixed-sex juvenile groups during adolescence showed less courtship and aggression towards unfamiliar conspecifics in a test situation as adults than males which grew up in mixed-sex juvenile pairs (Ruploh et al. 2013). It is not known, however, whether the behavioural and hormonal changes during adolescence are also similar to guinea pigs, which is the focus of the current study. Zebra finches are highly social, monogamous birds that typically live in large colonies of varying sizes (Zann 1996). In these colonies individuals often clump and allopreen, and intense aggression and territoriality normally only occur during breeding (Zann 1996). Breeding density can vary considerably between colonies and between individuals. Although most pairs breed within the main colony, others build their nests away from the main colony (Zann 1996, Mariette & Griffith 2013). The causes of this variation in sociality during breeding are not known.

Despite the lack of direct evidence, there are some indications that similar mechanisms might shape behaviour during adolescence in social birds as in social mammals. In zebra

finches, the adolescent period starts around post hatching day 35–40, at full nutritional independence. Sexual maturity is reached around day 60–90, yet morphological, behavioural and physiological maturation continues after sexual maturity (Zann 1996). Hence studies often use day 100–110 as the end of adolescence (Adkins-Regan & Leung 2006, Ruploh et al. 2013). When reared in isolation from day 31 to day 90 of age, zebra finch males show a significant delay in the development of adult sexual plumage traits compared to socially reared controls (Leader & Nottebohm 2006). Furthermore, rearing in social deprivation delays song development of zebra finch males (Morrison & Nottebohm 1993, Jones et al. 1996). In socially housed individuals, the start of the moult into the adult plumage occurs around day 35, together with the change in beak colour from black to red and a sensitive period for song learning associated with a peak in T (Pröve 1983, Adkins-Regan et al. 1990). This suggests that social stimulation may lead to developmental peaks in T or other hormones of the HPG axis which may then cause changes in morphological and behavioural transitions. As far as we know, there is no direct evidence yet for effects of social experiences during adolescence on developmental profiles of T.

It is also unclear whether the social environment during adolescence has similar effects on corticosterone (CORT) levels in zebra finches as in guinea pigs (Sachser et al. 2011). Social isolation of adult zebra finches results in increased CORT levels and reduced vocal activity (Perez et al. 2012). Moreover, experimental increases in CORT during early development have negative effects on adult courtship song (Spencer et al. 2003), suggesting that the social environment during adolescence may affect CORT with consequences for adult courtship behaviour.

To understand how the social environment during adolescence may affect adult behaviour in social birds, we experimentally investigated the behavioural, hormonal and morphological changes during adolescence and the resulting adult courtship and aggressive behaviour in male zebra finches by rearing them in three environments differing in social complexity. Earlier studies in zebra finches compared pairs and groups of juveniles and found similar effects on adult behaviour as studies in male guinea pigs using groups comprising both juveniles and adults (Sachser et al. 2011, Ruploh et al. 2013). Therefore and because in nature zebra finches also grow up with adults present in their environment (Zann 1996) we also wanted to study whether the presence of adults during adolescence modifies adult behaviour in zebra finches. We housed males in mixed-sex juvenile pairs (one juvenile male and one juvenile female; 1 m/1f), mixed-sex juvenile groups (three juvenile males and three juvenile females; 3 m/3 f), and mixed-sex, mixed-age groups (three juvenile males, three juvenile females, two adult males and two adult females; 5 m/5 f) to study the effects of group housing and the presence of adults in groups on social interactions and hormones during development, and later on adult traits.

Based on the studies in guinea pigs and earlier findings in zebra finches, we predicted that group housing would lead to increased social interactions and elevated or earlier peaks of T and CORT during adolescence. We also predicted that housing males in groups would lead to an accelerated development of song and plumage colouration. Finally, we predicted that group-reared males would show less courtship and aggressive behaviour as adults. Furthermore, we expected that the observed effects might differ between juvenile groups and mixed-age groups.

Methods

Subjects and housing conditions

The experimental subjects were initially 50 male domesticated zebra finches (*Taeniopygia guttata*), sired by 22 different breeding pairs at the University of Bielefeld. All males hatched in one of four aviaries located in the same indoor room with a controlled 14 h light:10 h darkness photoperiod (lights on at 7:00 h) with additional natural light entering through windows. Until adolescence, males lived together with their parents, peers and other adults and their offspring. Birds had ad libitum access to standard seed food (Elles, Mischfutter für Exoten, L. Stroetmann Saat, 48163 Münster, Germany) and water. Twice a week, this standard diet was enriched by egg food (Cédé N.V., 9940 Evergem, Belgium) and germinating seeds and once a week by fresh greens. Additionally, birds had access to a water bath 6 days a week. 12–16 days after hatching, individuals were given a black plastic ring with a unique identification number.

Social treatments

The experimental treatment started at the beginning of the adolescent period (average age 41 days; range: 36–45), when males were removed from their natal aviaries, and ended when the males were adult (average age 110 days, range: 104–114). This period was selected to ensure that the adolescent phase was covered until its end and all animals had reached sexual maturity (Zann 1996). Birds were either housed in juvenile pairs (one juvenile male and one juvenile female, 1 m/ 1 f), in juvenile groups (three juvenile males and three juvenile females, 3 m/ 3 f), or in mixed-age groups (three juvenile males, three juvenile females, two adult males and two adult females, 5 m/ 5 f). Siblings were never assigned to the same group and randomized over the different social treatments. Adults assigned to the mixed-age groups originated from our lab stock and were pairs that had already successfully bred with each other. They were unfamiliar to the juveniles. In total, we formed 14 juvenile pairs (n = 14 males), six juvenile groups (n = 18 males) and six mixed-age groups (n = 18

males). However, seven males had to be removed from their groups due to disease or incorrect initial sex assignment. Although four of these males were replaced within the first week by males of similar age (mean age difference between all males: 4 days; range: 1–9 days), all males removed and all replacement males were excluded from statistical analyses. This resulted in a final sample size of 43 subjects, comprising 10 males from juvenile pairs, 18 males from juvenile groups and 15 males from mixed-age groups. Each pair or group was housed in a small aviary (100 × 100 × 200 cm). In total, six different indoor rooms were used, each room containing two to three aviaries with a juvenile pair, one aviary with a juvenile group and one aviary with a mixed-age group. Treatment groups had no visual contact. All rooms had a controlled 14 h light:10 h darkness photoperiod (lights on at 7:00 h), using fluorescent full-spectrum light tubes (Osram, Biolux, L58W/965), and received no natural daylight. Birds had ad libitum access to seed food and water. This was supplemented twice a week by germinating seeds and once a week by fresh greens and a water bath. In all aviaries there were two feeders to minimize potential differences in food competition between groups of different sizes. At the beginning of the treatment period, all birds were symmetrically ringed with a second black ring and two identical colour rings. Assignment of colour rings was randomized within sex and age categories, and balanced across social treatments.

During adolescence, male social interactions and song were observed, T and CORT concentrations were measured, and the development of plumage colouration and weight was recorded. At the end of the adolescent period, males were individually housed and tested for their courtship and aggressive behaviour (Fig. 1).

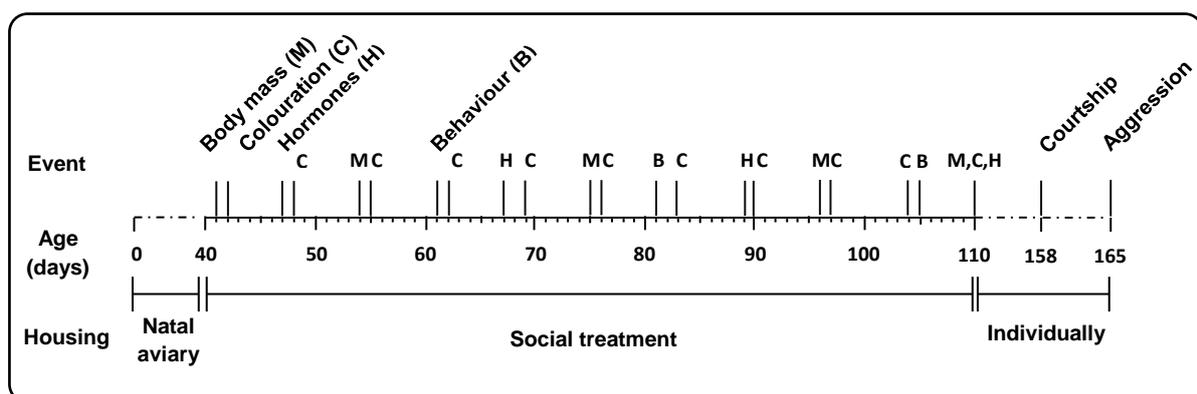


Fig. 1: Time line of experimental events during adolescence and in adulthood. The following experimental events took place: M: Body mass measurement; C: Colouration scan; H: Blood sampling for hormone analysis; B: Behavioural observations; Courtship: Courtship song test; Aggression: Aggressiveness test. For details on the number of subjects tested see results.

Behavioural observations

Behavioural observations of all 43 males were conducted three times during adolescence (Fig. 1) using focal animal sampling and continuous recording (Martin & Bateson 1993). Each male was observed twice on a given observation day for a 10-min interval, resulting in a total of 1 h of focal animal observations. Observations were performed between 8:00 h and 13:00 h by the same experimenter (SB), and all subjects within the same treatment room were observed on the same day. Observation intervals were separated by a break of 1.5–2 h, during which the other males in the same room were observed. The order of observations in a session was randomized for the different social treatments within a room, but males within the same aviary were observed in succession before moving on to the next aviary. To minimize disturbance of the birds, the observer observed from behind a screen. At the start of each observation interval, the observer waited for 2 min to allow startled birds to resume normal activities.

The recorded behavioural patterns were defined as follows:

Plastic song

A series of different vocal elements in variable and poorly structured order (Immelmann 1969, Scharff & Nottebohm 1991, Tchernichovski et al. 2001).

Song

A song starts with two or more identical, so-called introductory elements (Sossinka & Böhner 1980, Zann 1996). These are followed by a set of different vocal elements in a relatively fixed order (Sossinka & Böhner 1980, Zann 1996), which constitute a song motif. However, elements of a motif can be omitted or repeated, resulting in variable motif length (Sturdy et al. 1999, Honarmand et al. 2015). Several repeated motifs form a song (Zann 1996).

Plastic song and song were defined to end when the male was silent for at least two seconds, when a new song was started by introductory elements, or when the male stopped singing while hopping to another perch.

If sequences of elements were not repeated, they were not recorded since they could not easily be classified either as plastic song or song or other vocalisations (for recordings and spectrograms of different types of song during development see e.g. (Aronov et al. 2008)).

Social interactions

We recorded the following behaviours that involved direct physical interactions between a focal male and another individual as well as whether the behaviours were initiated by or directed towards the focal male:

Social exploration: One individual holds or pulls the feet, tail, or feathers of another individual with its beak or grabs food from the other's beak. Preening: One individual manipulates the feathers of another individual with its beak, performing a series of rapid movements (mostly on the head, but also on the back or sides). Pecking: One individual rapidly moves its beak towards another individual's body and touches it. Beak fighting: Two individuals peck at each other with their beaks, head-on or laterally, while in an upright posture. Chase: One individual flies towards another, followed by the latter's immediate displacement.

A social interaction ended when there was no more contact between individuals for two seconds or when the chased bird landed on a perch or the floor.

Since some behaviours were observed infrequently and not in all individuals, we summed up all social interactions for the analysis. Table 1 shows the frequencies of different behaviours recorded at each age.

Hormones

Blood samples were taken three times during adolescence (Fig. 1) to analyse T and CORT levels during development. Sample sizes for T differed between days because T was only measured if at least 15 μ l plasma was still available after CORT determination. Sample sizes for CORT analyses differed as not all samples could be obtained within 3 min after entering a treatment room (Wingfield et al. 1982, Romero & Reed 2005). It was not possible to repeat collection of these samples because that would have caused an unequal disturbance of different birds, thereby possibly affecting other experimental variables. Moreover, welfare considerations would have only allowed renewed sampling 1 week later. Blood samples were always collected between 11:00 h and 12:30 h to minimize circadian changes in T and CORT levels (Romero & Ramage-Healey 2000, Laucht et al. 2011). Each day, only males from one social treatment per room were sampled so that samples were taken over three successive days. Blood sampling was randomised for the social treatments across treatment rooms at each sampling session. Furthermore, we randomised the order of blood samples taken from males in different social treatments in the same room across sampling sessions. Birds were caught from the aviaries with a net and blood was collected in heparinised capillaries after puncturing the ulnar vein with a hypodermic needle. Capillaries were directly stored on ice. After a maximum of 1 h, plasma was separated by centrifugation (5000 rpm for 10 min) and frozen at -20°C until further processing.

T concentrations in plasma were determined in duplicate by enzyme immunoassay kits (DES 6622, Demeditec Diagnostics GmbH, Kiel, Germany) and then averaged. The antiserum used cross-reacted with relevant steroids as follows: testosterone 100%, 5 α -dihydrotestosterone 23.3%, androstenedione 1.6%, and all other tested steroids < 0.1%. The intra-assay coefficient of variation (CV) was 7.5% and the inter-assay CV was 9.1%. T concentrations were not detectable in 42 out of 150 samples. These 42 samples were assigned a value of zero for the analysis, to be conservative (assay sensitivity is 2.2 pg/ml, and samples had to be diluted between 3 and 44 times because variable amounts of blood were obtained during sampling). However, excluding these samples did not change the significance of the results or interpretation.

CORT concentrations were determined using corticosterone enzyme immunoassay kits (500655, Cayman Chemical, Michigan, USA) and were detectable in all samples. They were measured initially in duplicate, but if the % CV of the first duplicate measurement was higher than 15% and there was still sufficient plasma, they were measured in quadruplicate and subsequently averaged over all measurements. The antiserum used cross-reacted with relevant steroids as follows: corticosterone 100%, 11-dehydrocorticosterone 11%, 11-deoxycorticosterone 7%, progesterone 0.31%, cortisol 0.17%, and all other tested steroids < 0.1%. The intra-assay CV was 10.1% and the inter-assay CV was 10.6%.

Colouration

Males were visually scored once a week (Fig. 1) from outside the aviaries to quantify the development of the adult male colouration (Zann 1996, Leader & Nottbohm 2006). Traits scored included beak, cheek patches and breast stripes, with each trait scored separately for the left and the right body half of each male. Scores given ranged from 1 to 5 as follows: 1 (no colouration), 2 (less than 1/3rd coloured), 3 (between 1/3rd and 2/3rd coloured), 4 (more than 2/3rd coloured) and 5 (fully coloured) (Fig. 2). The separate scores on a given day were averaged for each subject for statistical analyses. The first colour score was not obtained for about half of the subjects due to time constraints when starting the experiment and the final score was not taken for one animal that was ill.

Body mass

During the treatment period, males were weighed five times (Fig. 1). For practical reasons, the first weighing took place between 10:00 h and 13:00 h of the day on which treatment groups were assigned. Subsequent weights were taken between 13:00 h and 18:00 h and within 2 h for all males housed in the same room. The first two weights were taken for only about half of the birds due to time constraints at the start of the experiment.

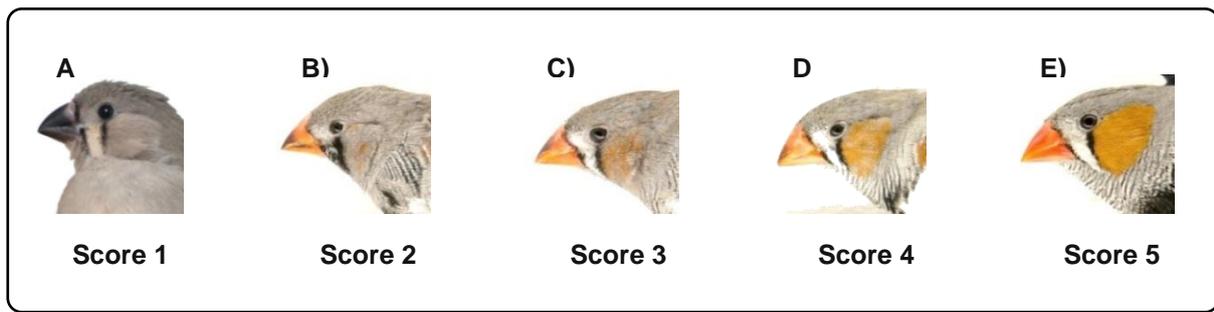


Fig. 2: Scores for the development of the male-typical colouration. Traits scored were beak, cheek patches and breast stripes. The pictures shown demonstrate cheek patch development as an example. A) Score 1 = no colouration of cheek patch; B) Score 2 = less than 1/3rd of cheek patch coloured; C) Score 3 = 1/3rd – 2/3rd of cheek patch coloured; D) Score 4 = more than 2/3rd of cheek patch coloured; E) Score 5 = complete colouration of cheek patch.

Adult housing

After the end of the social treatments males were housed individually in cages (30 cm high × 40 cm wide × 40 cm deep). We used the same protocol as previous experiments (Bischof et al. 2002, Ruploh et al. 2013, 2014a) to ensure that the design was comparable and that any long-term behavioural modifications could solely be explained by differences in social experiences during adolescence. Males were housed in the same room where they had hatched and had no visual, but auditory contact with each other and other birds in the room. Food and water was provided in the same manner as during the social treatment phase. On the transfer day, all rings were removed except the black numbered ring.

Courtship song test

Female-directed song (see definition of song) was quantified by presenting an unfamiliar female in a cage attached to the front of a male's cage. For each male, one of 16 different stimulus females was used whose presentation was randomized across males of different social treatments. We recorded the latency to start singing and the number of motifs produced in 10 min. Males that did not sing were assigned the maximum latency of 600 s and a motif rate of zero (6 pair-housed males, 3 juvenile group-housed males, and 6 mixed-age-group-housed males, a non-significant difference; $\chi^2 = 3.4$, $df = 2$, $p = 0.2$). Courtship song tests were performed between 9:00 h and 11:30 h in the males' home cages. The average age of males at courtship testing was 158 days (range: 152–161 days). One juvenile group male died shortly after the end of the social treatment phase, therefore only 42 males were included in the courtship song test.

Aggressiveness test

The aggressiveness test was performed 5–10 days after the courtship test for each individual (average age 165 days; range: 161–169 days). Male-male competition was tested in dyads by placing two males in a cage together with an unfamiliar female. Males were pseudo-randomly assigned to dyads of males from juvenile pairs and males from juvenile groups ($n = 4$), dyads of males from juvenile pairs and males from mixed-age groups ($n = 5$), and dyads of males from juvenile groups and males from mixed-age groups ($n = 7$). The remaining males were paired with replacement males or similar aged males that had remained in the natal breeding aviary. Initially, we included these males in the analysis but later decided against it because their number was too low for statistical analysis and their rearing conditions too different. Therefore the final sample size for the aggressiveness test was $n = 32$, with $n = 9$ for males from juvenile pairs, $n = 11$ for males from juvenile groups and $n = 12$ for males from mixed-age groups. In total, every male was tested once, in only one of the possible test combinations and brothers were never tested against each other.

A day before testing, the two males of a dyad were placed into a cage (30 cm high \times 80 cm wide \times 40 cm deep) which was divided by a non-transparent partitioning wall into two equally sized halves. Each half of the cage was equipped with two perches, a feeding station and a water dispenser. Males of a dyad were ringed with black or pink colour rings on both legs, with colours being equally allocated to males of different social treatments. To control for potential side effects during testing, initial placement of males into the right or left side of the cage was balanced across social treatments. Tests started the next morning by removing the partitioning wall between males and releasing a female into the cage. Subsequently, the number of chases males performed towards each other was recorded as a measure of aggressiveness. The 16 stimulus females used for the aggressiveness test were the same ones that had been used for the courtship song test, but they were always unfamiliar to both males of a tested dyad. Each test lasted 1 h. Test sessions started between 8:00 h and 9:00 h and ended between 12:00 h and 13:00 h, as four male dyads were tested consecutively. Aggression tests were performed in the males' standard housing room.

Data analysis

Some data were transformed to achieve equal variances between social treatment groups and normally distributed residuals. Variances and distributions were assessed visually using variance plots, histograms of residuals and Q-Q plots. A square root transformation was used for plastic song, song motifs and chases (n/min). A log₁₀ transformation was used for T and CORT concentrations (ng/ml), after adding a value of one to each measure. Body mass, colouration, and the courtship song latency met the criteria of variance homogeneity and

Table 1: Experimental measures during adolescence

A)	Behaviour/min	Age at observation: 61 days					Age at observation: 81 days					Age at observation: 105 days							
		Directed from focal male to:				all	per	Directed from focal male to:				all	per	Directed from focal male to:				all	per
		juv ♀	juv ♂	ad ♀	ad ♂	partners	partner	juv ♀	juv ♂	ad ♀	ad ♂	partners	partner	juv ♀	juv ♂	ad ♀	ad ♂	partners	partner
Juvenile pairs	Social exploration	0.050				0.050	0.050	0.010				0.010	0.010	0.015				0.015	0.015
	Preening	0.035				0.035	0.035	0.010				0.010	0.010	0.035				0.035	0.035
	Pecking	0.010				0.010	0.010	0.025				0.025	0.025	0.045				0.045	0.045
	Beak fighting	0.005				0.005	0.005	0.000				0.000	0.000	0.010				0.010	0.010
	Chasing	0.000				0.000	0.000	0.000				0.000	0.000	0.000				0.000	0.000
Juvenile groups	Social exploration	0.042	0.022			0.064	0.013	0.039	0.019			0.058	0.012	0.042	0.028			0.070	0.014
	Preening	0.044	0.025			0.069	0.014	0.042	0.008			0.050	0.010	0.022	0.008			0.030	0.006
	Pecking	0.014	0.019			0.033	0.007	0.031	0.025			0.056	0.011	0.047	0.011			0.058	0.012
	Beak fighting	0.003	0.003			0.006	0.001	0.006	0.003			0.009	0.002	0.031	0.003			0.034	0.007
	Chasing	0.000	0.000			0.000	0.000	0.000	0.000			0.000	0.000	0.000	0.000			0.000	0.000
Mixed-age groups	Social exploration	0.040	0.020	0.037	0.013	0.110	0.012	0.020	0.010	0.000	0.013	0.043	0.005	0.010	0.007	0.013	0.003	0.033	0.004
	Preening	0.027	0.037	0.007	0.007	0.078	0.009	0.063	0.007	0.003	0.000	0.073	0.008	0.010	0.010	0.000	0.000	0.020	0.002
	Pecking	0.013	0.007	0.013	0.000	0.033	0.004	0.017	0.010	0.010	0.010	0.047	0.005	0.037	0.007	0.017	0.007	0.068	0.008
	Beak fighting	0.007	0.003	0.000	0.010	0.020	0.002	0.000	0.003	0.003	0.000	0.006	0.001	0.000	0.000	0.000	0.000	0.000	0.000
	Chasing	0.000	0.000	0.003	0.003	0.006	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.023	0.003
B)	Behaviour/min	Directed to focal male from:				all	per	Directed to focal male from:				all	per	Directed to focal male from:				all	per
		juv ♀	juv ♂	ad ♀	ad ♂	partners	partner	juv ♀	juv ♂	ad ♀	ad ♂	partners	partner	juv ♀	juv ♂	ad ♀	ad ♂	partners	partner
Juvenile pairs	Social exploration	0.085				0.085	0.085	0.025				0.025	0.025	0.020				0.020	0.020
	Preening	0.020				0.020	0.020	0.005				0.005	0.005	0.040				0.040	0.040
	Pecking	0.010				0.010	0.010	0.020				0.020	0.020	0.055				0.055	0.055
	Beak fighting	0.005				0.005	0.005	0.010				0.010	0.010	0.015				0.015	0.015
	Chasing	0.000				0.000	0.000	0.000				0.000	0.000	0.000				0.000	0.000
Juvenile groups	Social exploration	0.019	0.047			0.066	0.013	0.061	0.014			0.074	0.015	0.039	0.042			0.081	0.016
	Preening	0.039	0.011			0.050	0.010	0.047	0.047			0.094	0.019	0.044	0.008			0.052	0.010
	Pecking	0.017	0.008			0.025	0.005	0.033	0.039			0.072	0.014	0.036	0.014			0.050	0.010
	Beak fighting	0.014	0.006			0.020	0.004	0.000	0.000			0.000	0.000	0.011	0.000			0.011	0.002
	Chasing	0.000	0.000			0.000	0.000	0.003	0.000			0.003	0.001	0.000	0.000			0.000	0.000

Table 1: Experimental measures during adolescence (*continued*)

Mixed-age groups	Social exploration	0.050	0.047	0.013	0.003	0.113	0.013	0.060	0.020	0.003	0.000	0.083	0.009	0.037	0.010	0.003	0.000	0.050	0.006
	Preening	0.020	0.047	0.007	0.000	0.074	0.008	0.023	0.030	0.000	0.000	0.053	0.006	0.007	0.010	0.003	0.000	0.020	0.002
	Pecking	0.000	0.017	0.040	0.013	0.070	0.008	0.030	0.023	0.043	0.013	0.109	0.012	0.027	0.020	0.027	0.003	0.077	0.009
	Beak fighting	0.003	0.003	0.000	0.007	0.013	0.001	0.000	0.000	0.003	0.000	0.003	0.001	0.007	0.007	0.003	0.000	0.017	0.002
	Chasing	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Given are the mean age of subjects and the frequencies of different behaviours towards different interaction partners recorded at each observation during adolescence. Behaviours a) initiated and b) received by focal males were social exploration, preening, pecking, beak fighting and chasing. Interaction partners: juv. ♀: juvenile female; juv. ♂: juvenile male; ad. ♀: adult female; ad. ♂: adult male

normally distributed residuals and were thus analysed using the untransformed values. Social interactions (n/min) during development were Poisson distributed and analysed by a generalised linear mixed model (GLMM) using a Poisson distribution. All other data were analysed by linear mixed models (LMM), assuming a normal distribution.

Data were analysed in R 2.13.1 by mixed effects models using a maximum likelihood approach (package lme4). Significances of effects were calculated using likelihood ratio tests. Effects with a $p > 0.1$ were removed stepwise from the model. The highest order interactions were always tested first, followed by lower order interactions, until the final model was obtained. Whenever higher order interactions were significant, all lower order interactions remained in the model. Main effects of social treatment and age were always kept in the model. Subsequent pairwise comparisons between two experimental treatments were conducted using Sidak adjustments to account for multiple testing.

The analyses included a random effect of the experimental aviary (“treatment group ID”), a random effect of the male’s family (“nest ID”), and a random effect of the male’s identity (“male ID”) for data with multiple measures of each male. “Nest ID” and “male ID” were kept in all models to control for non-independence of multiple measures from the same male and from brothers allocated to different treatment groups. A random effect of “treatment group ID” was removed from the models if it was clearly not statistically significant ($p > 0.2$).

To analyse the differences in development between the three social treatments, the effects of treatment (“social treatment”), age at testing (“age”) and their interaction were tested. Since the change with age in most traits was non-linear, we included the effects of higher order polynomials of “age”, which is a frequently used method to model non-linear slopes in a mixed-modelling frame work (Goldstein 2003). We included up to the 5th polynomial of age if significant. As the effect of the social treatment is likely to be weaker at the start of the experiment and might affect the birds more at certain ages than others, we expected to find significant interactions between treatment and age. In most cases, an interaction with a polynomial of age was significant. In some cases, only the main effect of the polynomial of age was significant and hence stayed in the model (for corticosterone: age³; body mass: age³; colouration: age⁵).

Adult behaviour in the courtship song test was analysed with “social treatment” as the only fixed factor. Adult behaviour in the aggressiveness test was analysed with “social treatment” and type of opponent (“opponent treatment”) as fixed factors. Since males were never tested with an opponent from their own social treatment, post-hoc comparisons of experimental groups could not be performed on the interaction of “social treatment” and “opponent”. Therefore, we only tested for a main effect of opponent by choosing one experimental group and “opponent” as the only fixed factor. Analyses for an effect of opponent were conducted using linear models (LM), as there were no random effects. Since a normal distribution could

not be achieved by any transformation of motif rate or rate of chases, these data were also analysed non-parametrically using Kruskal-Wallis tests and pairwise Wilcoxon tests. This did not change the significance of any result, therefore only the parametric statistics are presented in the figures and text.

Graphs show means \pm standard error (SE) estimated with “social treatment”, “age”, and “opponent treatment” as categorical factors, including the random effects. For models including age as a factor, graphs also show the prediction lines from the final models. The significance level α was set at $p < 0.05$.

Results

Social interactions

The interaction of treatment and age had a significant effect on the frequency of social interactions initiated by the focal males (GLMM: social treatment x age: $\chi^2 = 7.0$, $df = 2$, $p = 0.03$; Fig. 3A) and on the frequency of social interactions directed by other birds towards the focal males (GLMM: social treatment x (age+age²): $\chi^2 = 19.9$, $df = 4$, $p < 0.001$; Fig. 3A).

When comparing pairs of experimental groups, males from mixed-age groups and males from juvenile groups differed with regard to how the social interactions they initiated changed as they matured (GLMM: social treatment x age: $\chi^2 = 6.8$, $df = 1$, $p < 0.03$). The same was found for the social interactions they received (GLMM: social treatment x (age+age²): $\chi^2 = 8.4$, $df = 1$, $p < 0.05$). Mixed-age group males had most interactions during early adolescence, but these decreased towards late adolescence. In juvenile groups, social interactions slightly increased from early to late adolescence. Males from both group conditions also initiated on average more social interactions than males from juvenile pairs (GLMM: social treatment: $\chi^2 > 7.2$, $df = 1$, $p < 0.02$; to test whether there was an overall difference in social interactions we removed the effect of the interaction of social treatment and age for this analysis). Furthermore, males from both group treatments differed significantly from males in juvenile pairs with regard to how the social interactions directed towards them changed as they matured (GLMM: social treatment x (age+age²): $\chi^2 > 9.7$, $df = 1$, $p < 0.02$). In juvenile pairs, social interactions directed towards males initially decreased, but then increased again. The opposite pattern was seen in group-housed males.

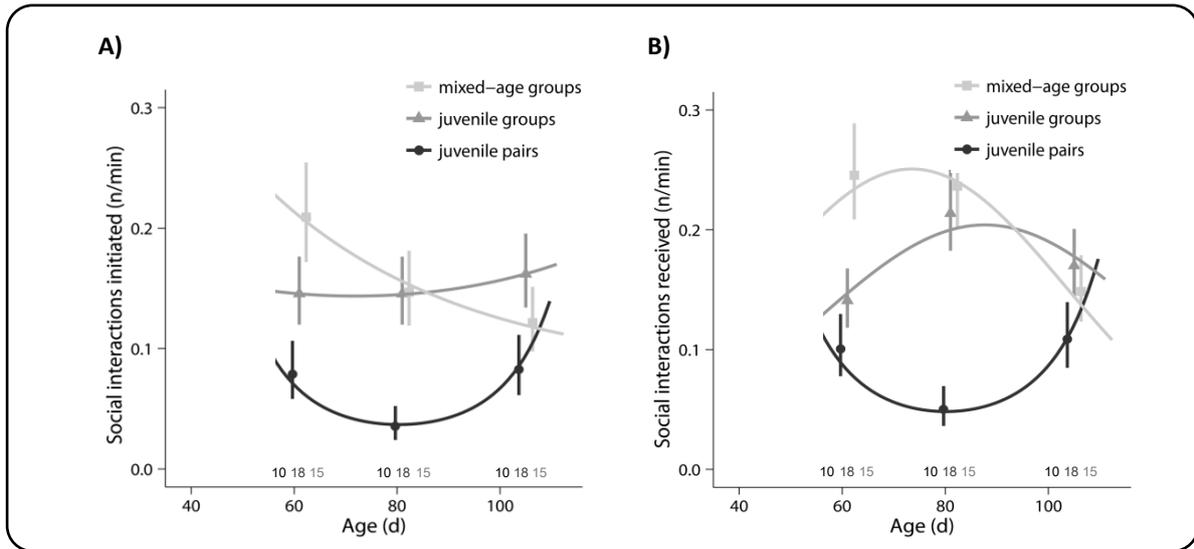


Fig. 3: Social interactions during adolescence. Males reared in groups **A)** initiated more social interactions than males from juvenile pairs and **B)** differed from them with regard to how received social interactions changed as they matured. Furthermore, group males differed from each other with regard to how the frequency of interactions **A)** initiated and **B)** received changed during development. For details, see text. Shown are means \pm SE for each age and lines from the model with the best fit. Sample sizes are shown directly above the x-axis.

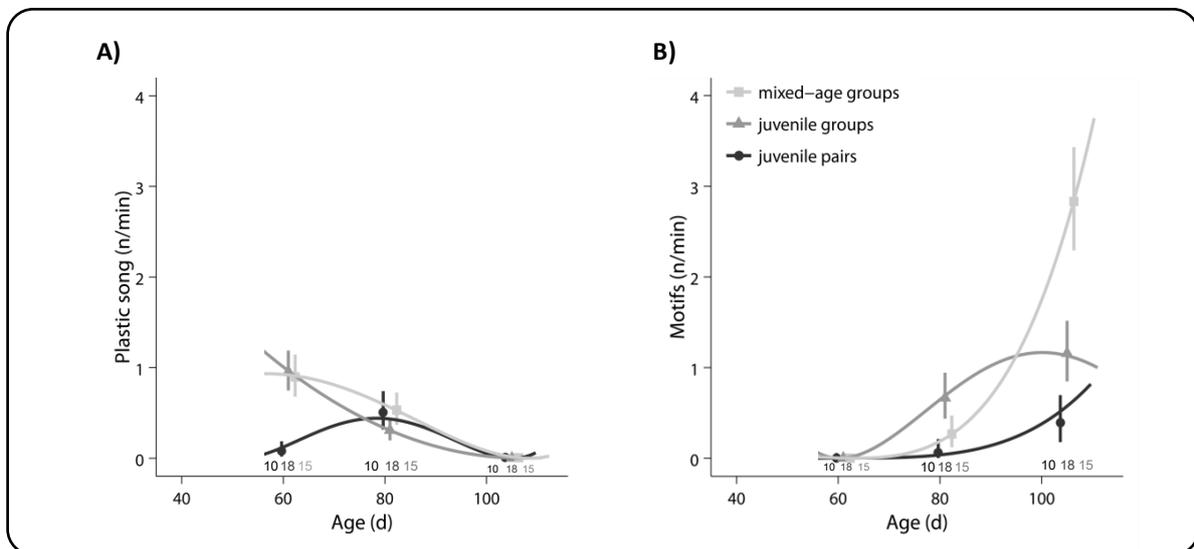


Fig. 4: Plastic song and song during adolescence. **A)** Group males showed higher rates of plastic song than pair males during early adolescence. **B)** Mixed-age group males showed a higher increase in song motif rate than juvenile group males and juvenile pair males in late adolescence. For details, see text. Shown are means \pm SE for each age and lines from the model with the best fit. Sample sizes are shown directly above the x-axis.

Plastic song and song

The social environment experienced during adolescence significantly influenced how male plastic song (Fig. 4A) and song (Fig. 4B) changed during development (LMM for song: social treatment x (age+age²): $\chi^2 = 16.7$, df = 4, p = 0.002; LMM for plastic song: social treatment x (age + age²): $\chi^2 = 17.5$, df = 4, p = 0.002). Males from mixed-age groups differed significantly from males reared in juvenile pairs in the development of plastic song (LMM: social treatment x (age + age²): $\chi^2 = 10.4$, df = 2, p < 0.02). The same was true for males from juvenile groups (LMM: social treatment x (age + age²): $\chi^2 = 14.3$, df = 2, p = 0.002). There was no significant difference between the group treatments in plastic song development (LMM: social treatment x (age + age²): $\chi^2 = 1.3$, df = 2, p > 0.9). Group-reared males showed high rates of plastic song during early adolescence when pair-reared males produced almost no plastic song. The frequency of plastic song was similar during late adolescence when pair-reared males started producing plastic song.

The change of song with age differed significantly between males reared in mixed-age groups and those reared in juvenile groups (LMM: social treatment x (age + age²): $\chi^2 = 11.9$, df = 2, p < 0.01). Furthermore, the change of song with age differed significantly between males reared in mixed-age groups and males reared in juvenile pairs (LMM: social treatment x (age + age²): $\chi^2 = 9.6$, df = 2, p = 0.024). Juvenile group males and juvenile pair males did not differ significantly in song development (LMM: social treatment x (age + age²): $\chi^2 = 4.7$, df = 2, p = 0.26). Males from mixed-age groups increased song motif rate most strongly towards late adolescence. Males from juvenile groups showed a more constant moderate increase in motif rate from early to late adolescence. Finally, males from pairs showed a slight increase in motif rate only in late adolescence.

Hormones

Testosterone

There was a significant effect of the interaction between social treatment and the cubic part of the curve fit (LMM: social treatment x age³: $\chi^2 = 6.8$, df = 2, p = 0.03; Fig. 5), indicating that the social environment during adolescence influenced plasma T profiles. However, the difference in the overall temporal profile in T did not reach significance (LMM: social treatment x (age + age² + age³): $\chi^2 = 10.0$, df = 6, p = 0.1). T profiles of males from mixed-age groups and males from juvenile groups differed significantly in the cubic part of the curve fit (LMM: social treatment x age³: $\chi^2 = 6.1$, df = 1, p = 0.04). There was no significant difference in T profiles (LMM: social treatment x age³: $\chi^2 < 4.5$, df = 1, p > 0.1) or average

T levels (LMM: social treatment: $\chi^2 < 1.9$, $df = 1$, $p > 0.1$) between the other treatments. Males from juvenile groups showed a pronounced peak in T in late adolescence. In mixed-age groups T levels increased only slightly in early adolescence and again in late adolescence and there was no peak at any age. Males from juvenile pairs showed a slight decrease in T in early adolescence and a moderate increase in late adolescence.

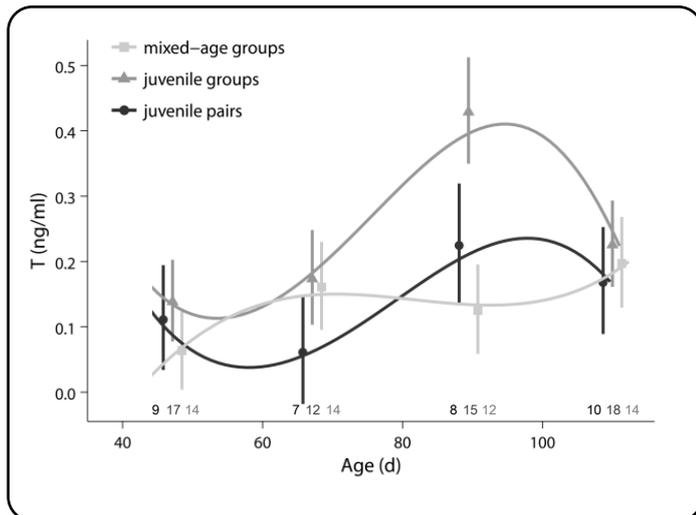


Fig. 5: Testosterone levels during adolescence. Juvenile group males had a pronounced peak in testosterone (T) in late adolescence, which was missing in males from mixed-age groups. For details, see text. Shown are means \pm SE for each age and lines from the model with the best fit. Sample sizes are shown directly above the x-axis.

Corticosterone

Plasma CORT profiles were not affected by the social environment during adolescence (social treatment \times age: $\chi^2 = 2.4$, $df = 2$, $p = 0.3$; social treatment: $\chi^2 = 0.5$, $df = 2$, $p = 0.8$; Fig. 6). All males showed a significant decline in plasma CORT with age (age + age² + age³: $\chi^2 = 38.6$, $df = 3$, $p < 0.001$).

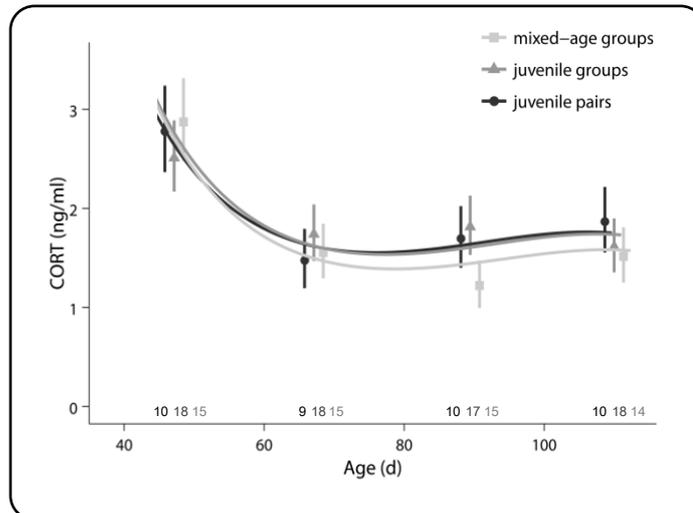


Fig. 6: Corticosterone levels during adolescence. Corticosterone (CORT) profiles of males during adolescence did not differ between treatments. For details, see text. Shown are means \pm SE for each age and lines from the model with the best fit. Sample sizes are shown directly above the x-axis.

Colouration

The development of the male-typical colouration during adolescence differed significantly between males from different social rearing environments (LMM: social treatment \times (age+age²+age³): $\chi^2 = 23.7$, $df = 6$, $p < 0.001$; Fig. 7). Males from mixed-age groups developed the characteristics of the adult male plumage significantly faster than males from juvenile pairs (LMM: social treatment \times (age+age²+age³):

$\chi^2 = 16.4$, $df = 3$, $p < 0.01$). In addition, males from mixed-age groups developed the adult plumage traits significantly faster than males from juvenile groups (LMM: social treatment \times (age+age²+age³): $\chi^2 = 14.1$, $df = 3$, $p < 0.01$). Juvenile group males and juvenile pair males did not differ in the development of plumage colouration (LMM: social treatment \times (age+age²+age³): $\chi^2 = 4.5$, $df = 3$, $p = 0.5$).

Body mass

Although statistically not significant, the increase in weight with age during adolescence tended to differ between males from different social rearing environments (LMM: social treatment \times age: $\chi^2 = 4.8$, $df = 2$, $p = 0.09$; Fig. 8). Males that grew up in mixed-age groups tended to gain more weight than males that grew up in juvenile pairs, especially during late adolescence (LMM: social treatment \times age: $\chi^2 = 5.1$, $df = 1$, $p = 0.07$). There was no difference between the other treatments (LMM: social treatment \times age: $\chi^2 < 2.3$, $df = 1$, $p > 0.3$).

Courtship song test

In the courtship song test in adulthood, there was a significant difference in the latency to start the first song between males from different social-rearing environments (LMM: social

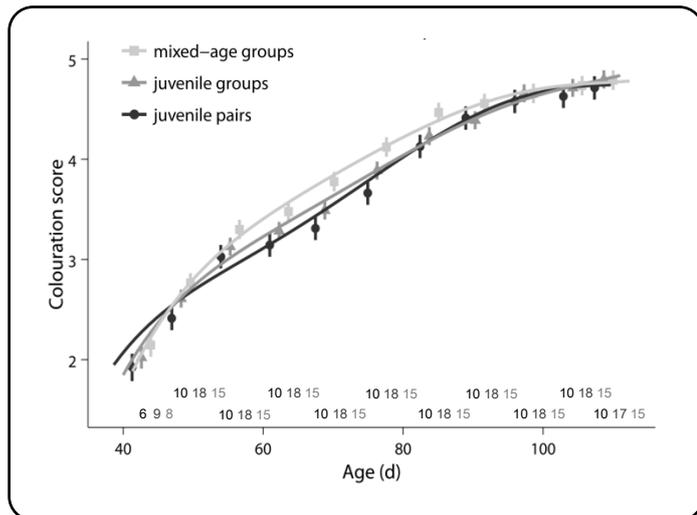


Fig. 7: Plumage colouration during adolescence. Males from mixed-age groups developed the adult colouration significantly faster than males from juvenile pairs and males from juvenile groups. For details, see text. Shown are means \pm SE for each age and lines from the model with the best fit. Sample sizes are shown directly above the x-axis.

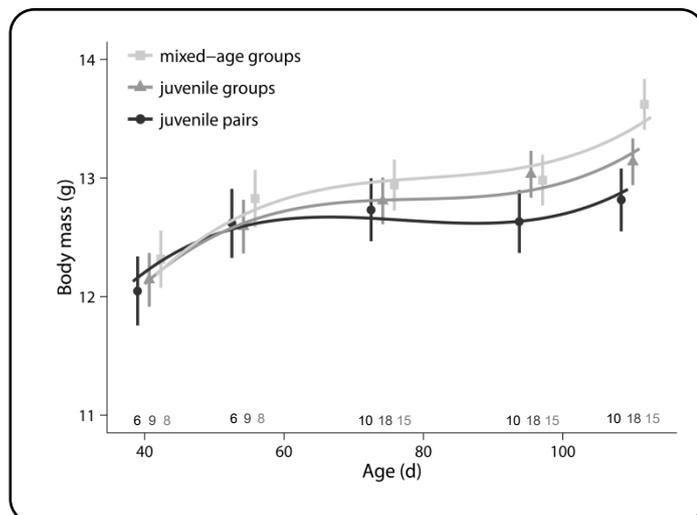


Fig. 8: Body mass during adolescence. Males from mixed-age groups tended to gain more weight during adolescence than males from juvenile pairs. For details, see text. Shown are means \pm SE for each age and lines from the model with the best fit. Sample sizes are shown directly above the x-axis.

treatment: $\chi^2 = 8.9$, $df = 2$, $p = 0.01$; Fig. 9A). In addition, motif rates tended to differ between social treatments (LMM: social treatment: $\chi^2 = 5.8$, $df = 2$, $p = 0.06$; Fig. 9B). Males from juvenile groups started to sing significantly faster than males from juvenile pairs (LMM: social treatment: $\chi^2 = 9.3$, $df = 1$, $p = 0.007$). Furthermore, they showed a tendency to sing more motifs per minute than pair males. (LMM: social treatment: $\chi^2 = 5.8$, $df = 1$, $p = 0.05$). The other treatments did not differ significantly in singing latency (LMM: social treatment: $\chi^2 < 3.0$, $df = 1$, $p > 0.24$) or motif rate (LMM: social treatment $\chi^2 < 2.7$, $df = 1$, $p > 0.27$).

Aggressiveness test

There was a significant effect of the interaction between social treatment and opponent treatment on the rate of chases initiated by the focal males in adulthood (LMM: social treatment x opponent: $\chi^2 = 7.1$, $df = 1$, $p < 0.008$; Fig. 10A). The same was true for the rate of chases directed by other birds towards the focal males (LMM: social treatment x opponent: $\chi^2 = 8.3$, $df = 1$, $p < 0.004$; Fig. 10B). Post-hoc tests revealed that males from juvenile groups (LM: t-value = 6.0, $p < 0.001$) and males from mixed-age groups (LM: t-value = 2.5, $p = 0.03$) showed a significantly higher rate of aggression towards males from juvenile pairs than towards males from the other group treatment. In contrast, males from juvenile pairs showed similarly low rates of aggression towards both males from juvenile groups and males from mixed-age groups (LM: t-value = 1.5, $p = 0.19$). Further post-hoc tests on the aggression directed towards focal males confirmed that males reared in juvenile pairs received most aggression and males reared in groups received least aggression (Fig. 10B, analysis not shown).

Discussion

In recent years, more and more studies have found long-lasting effects of the adolescent social environment on adult behaviour, yet we still know very little about the underlying behavioural and physiological mechanisms. We describe for the first time in zebra finches how the size and age composition of social groups during adolescence affect social interactions, song development, plumage colouration and T concentrations with long-lasting consequences for adult courtship and aggressive behaviour. Our results suggest that the effects of the social environment on adult behaviour may be mediated by differences in behavioural and physiological maturation.

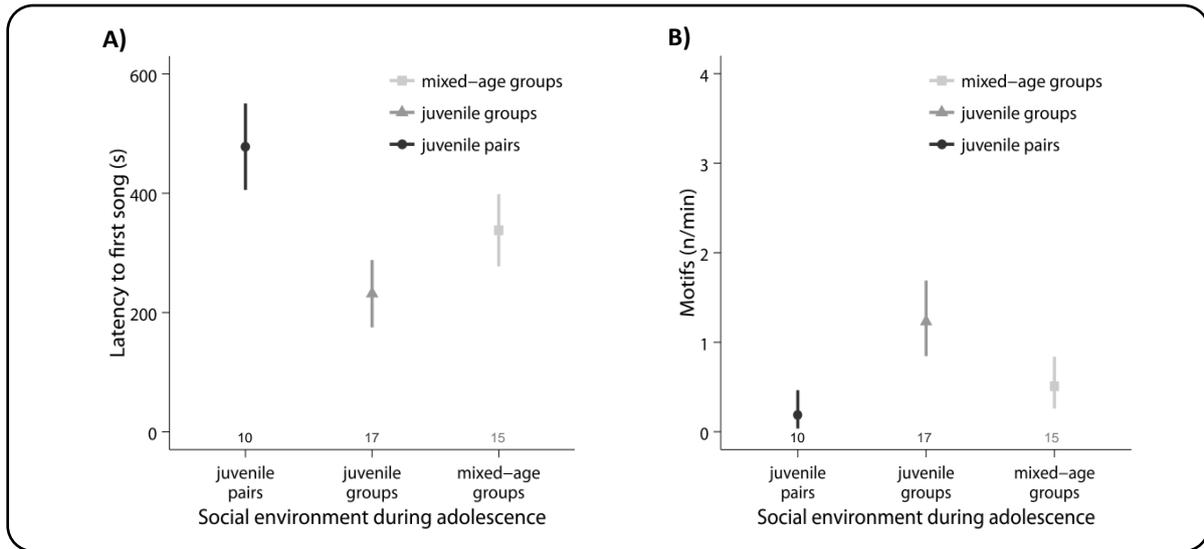


Fig. 9: Courtship singing in adulthood. Males from juvenile groups **A)** started to sing significantly faster than males from juvenile pairs and **B)** showed a tendency to sing with a higher motif rate than males from juvenile pairs. For details, see text. Shown are means \pm SE for each age. Sample sizes are shown directly above the x-axis.

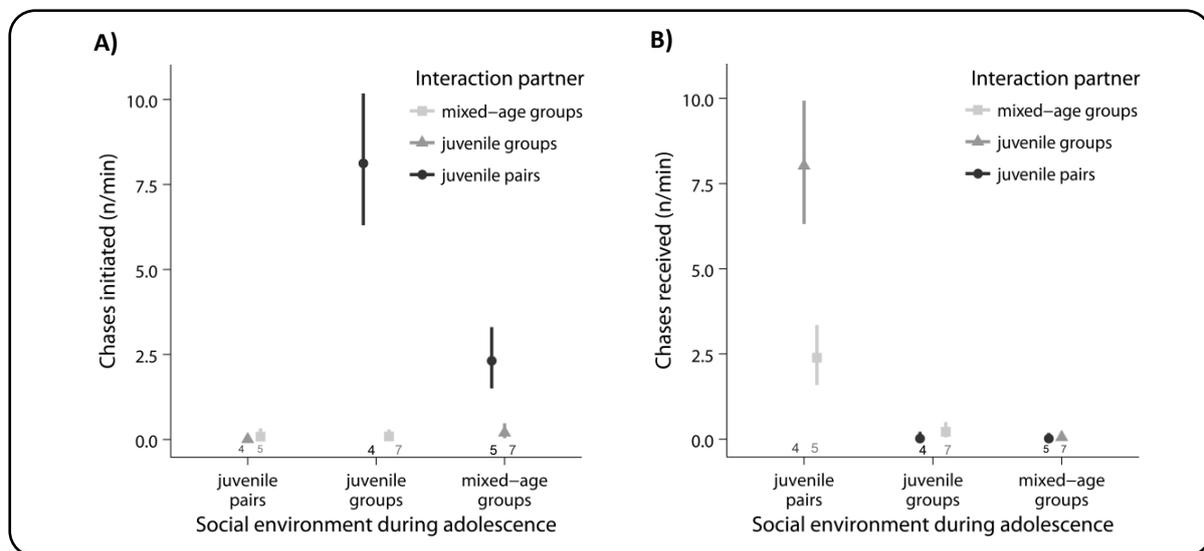


Fig. 10: Aggressiveness in adulthood. **A)** Males from groups initiated more chases when their opponent was from a juvenile pair than when he was from the other group treatment, whereas males from juvenile pairs directed few chases towards males from either group treatment. **B)** Males from groups received fewer chases than males from juvenile pairs from all interaction partners. For details, see text. Shown are means \pm SE for each age. Sample sizes are shown directly above the x-axis.

Maturation and adult behaviour

Group housing during adolescence enhanced the frequency of social interactions, song development, plumage colouration and the frequencies of courtship and aggressive behaviour in adulthood. Although some effects were more pronounced in juvenile groups, others were stronger in mixed-age groups. Earlier studies on zebra finches found that social isolation delayed song development (Morrison & Nottebohm 1993, Jones et al. 1996) and plumage maturation (Leader & Nottebohm 2006). Our results suggest that this is not an artefact of extreme social deprivation, but that male zebra finches can mature more rapidly at higher social densities. Rapid maturation at high densities may be beneficial if higher densities indicate a larger variety of potential partners or, more generally, a high quality environment that is more suitable for reproduction. Since zebra finches are monogamous, adult males do not monopolise females at high densities. In the polygynous guinea pig by contrast, males reared at high densities are thought to follow a queuing strategy associated with low aggression and courtship to avoid competition with adult males (Sachser et al. 2011). In the wild, maturation in zebra finches is also affected by the early environment, as males born early in the season develop adult plumage more rapidly and can breed at a much younger age than males born late in the season, the latter delaying plumage maturation and breeding until the next season (Zann 1996). Even though zebra finches are opportunistic breeders with early sexual maturation, they nevertheless appear to be able to benefit from adjusting reproductive investment to social and ecological conditions (Perfito et al. 2007, Perfito 2010).

Group-housing not only stimulated maturation, but also increased adult courtship behaviour, which may be beneficial when reproductive opportunities increase. In zebra finches, females prefer e.g. males with a high motif rate (Collins et al. 1994). In addition, group housing increased aggressiveness, but only towards pair-housed males. This suggests that the appearance or behaviour of the opponent is crucial in stimulating higher aggressiveness. Similar effects have been described in male Syrian golden hamsters where the experience of losing in agonistic interactions with adult males can result in enhanced levels of aggression during adulthood (Ferris et al. 2005), especially when confronted with inferior opponents (Delville et al. 1998). This might explain why males reared only with a juvenile female received most aggression: males reared in pairs may trigger more aggression in group-reared males as they have not learned to display appropriate social behaviour towards same sex opponents and hence appear less socially skilled or inferior to the more competent group-reared males. Importantly, the effects on adult courtship and aggression contrasted with earlier studies on the influence of the adolescent social environment on adult behaviour in zebra finches (Ruploh et al. 2013, 2014a), which will be discussed further below.

Differences between groups with and without adults

Some effects of group housing on behaviour and physiology differed between juvenile groups and mixed-age groups. We cannot disentangle whether these effects are due to group composition, group size or housing density because mixed-age groups were larger than juvenile groups and aviaries had the same size so that less space was available per individual. Also, males in mixed-age groups had nearly twice as many interaction partners than those in juvenile groups, but not twice as many social interactions, and during late adolescence they had even fewer social interactions. We therefore do not know whether the observed effects are due to an increase in social interactions, due to a larger number of interaction partners or possibly even due to a reduction in social interactions with each individual in the group. However, we presume that it is more likely that the observed differences are due to the presence of adults rather than group size or density. High densities often have negative effects on body mass (Poot et al. 2012), but in this experiment and previous studies in our lab (Ruploh et al. 2014a), there were no significant differences in body mass between experimental groups. In contrast, the presence of adults during development has been found to influence the frequency and type of interactions juveniles' experience in several species, with species-specific consequences for their maturation and adult behaviour (West & King 1988, White et al. 2002, Wommack et al. 2003, Arnold & Taborsky 2010, Sachser et al. 2013). Interestingly, the presence of adults can have different effects depending on the age during which adolescents interact with them (Delville et al. 2003) and the context in which they are tested as adults (Delville et al. 1998, Arnold & Taborsky 2010). Thus, the presence of adults can both stimulate and inhibit sexual maturation of young, which may explain some of our results. If we split the experimental phase that lasted from shortly after nutritional independence to early adulthood (day 41 to day 110) into two periods, we can distinguish between early adolescence (~day 41–75) and late adolescence (~day 76–110). We choose this division because sexual maturity is attained between day 60–90 in zebra finches (Zann 1996), ~day 75 being the median age. Early adolescence thereby represents the time period before the first reproductive event, while late adolescence represents the period during which all birds reach sexual maturity. During early adolescence, the presence of adults might have a positive effect because young zebra finches and other birds are e.g. attracted to adult males to learn song (Immelmann 1969, Eales 1985, Clayton 1987, Williams 1990, Freeberg 2000, Catchpole & Slater 2008). Therefore in our study, the presence of adults might have increased social interactions, thereby stimulating plumage maturation in early adolescence. As a result, mixed-age group males might have moulted into the adult plumage faster than juvenile pair males or juvenile group males.

In contrast, during late adolescence when males are close to sexual maturity, interactions with adults may occur in a context of competition and reproduction. Zebra finches are monogamous and males and females guard their mates against approaches by same-sex rivals (Zann 1996). Especially in juveniles the frequency of courtship attempts towards mated individuals is high (Zann 1996). We suggest that through the behavioural feedback of interaction partners juveniles learn about the discrimination of interaction partners that are already paired or still available. Unfortunately, we did not have enough behavioural data to analyse sexual and aggressive behaviour with different interaction partners during adolescence. However, interactions with adults can accelerate and improve sexual behaviour of young during development and adulthood in other species (West & King 1988, Delville et al. 2003, Wommack et al. 2003, Miller et al. 2008). Juvenile males in mixed-age groups may have had more opportunities to learn and fine-tune their behaviour, which could explain the increase in singing rate during late adolescence in mixed-age group males. However, in adulthood, courtship singing was highest in juvenile group males, not in mixed-age group males. This may be because in mixed-age groups juvenile males have learned that they are less successful in courtship due to the presence of more attractive and competitive adult males and may therefore have been inhibited by their previous experiences. Zebra finch males that are less successful in pairing with female conspecifics as juveniles also have reduced pairing success later in life (Mariette et al. 2013). While the frequency of social interactions in mixed-age group males decreased from early to late adolescence, the opposite was seen in juvenile group males. We suggest that males in juvenile groups initially interacted less due to their smaller group sizes or the absence of adults during early adolescence, but they increased social interactions when approaching sexual maturity as they were not inhibited by older, more experienced males.

In conclusion, social interactions, song and plumage developed differently in groups with adults than in those without adults, but there was little evidence of differences in adult behaviour. This suggests that the most striking, long-lasting effects of the social environment during adolescence on adult courtship and aggression observed in zebra finches in this and earlier studies (Ruploh et al. 2013, 2014a) may not primarily depend upon the age structure of groups.

The role of endocrine changes

The observed differences in plumage maturation and behaviour may partly be due to endocrine changes. During early adolescence, plumage maturation was enhanced in groups with adults. At that age there were no differences in T levels, therefore we have to consider other hormones that are involved in social interactions and regulate plumage colouration

(Ralph 1969, Harding 1981, Wingfield 1994, McGraw 2006, Ramage-Healey et al. 2008). In many avian species, estradiol and luteinizing hormone (LH) are important regulators of plumage colouration (Ralph 1969, McGraw 2006) and thus should be measured in future studies. Although LH stimulates the secretion of T from the gonads, the gonads may still be insensitive to LH during early adolescence (Odell et al. 1974) which could explain why we found little evidence for differences in T levels. Plumage development may also be related to nutritional differences (Naguib & Nemitz 2007), although the higher weight gain of mixed-age group males compared to juvenile pair males during adolescence did not reach significance. Moreover, it has been shown that interactions between hormones and nutrition result in differences in plumage colouration, as hormones affect the deposition of pigments from food items in the feathers (Ralph 1969, McGraw 2006).

In contrast to plumage differences, some behavioural differences between males during development and in adulthood may directly reflect differences in T levels. Juvenile group males did experience a T peak in late adolescence, as reported earlier for this stage of life in socially housed zebra finch males (Pröve 1983). This peak was absent in mixed-age group males, most likely due to an inhibiting effect of the adults present in the groups (e.g. Vandenberg 1971, Bushmann & Burns 1994). As the T peak normally occurring in late adolescence coincides with a sensitive period for song learning (Pröve 1983) and the absence of T or an excess of T impairs the crystallization of song in zebra finches and other songbirds to its final stable form (Bottjer & Johnson 1997, Schlinger 1997), this might explain why mixed-age group males still increased song rates during that time. Their song rates (ca. 3 motifs/ min) are more than tenfold higher than song rates previously observed in captivity or in the wild (less than 0.2 motifs/min, (West & King 1988, Dunn & Zann 1996), and more similar to song rates seen during the first 10–15 min when males first encounter unfamiliar females (ca. 1–4 motifs/min (Ruploh et al. 2013, 2014a)). In contrast, elevated T in juvenile groups may have inhibited further changes in song during late adolescence.

Although T concentrations and song development during adolescence differed significantly between juvenile groups and mixed-age groups, adult courtship and aggression did not differ significantly between them. At the same time, T concentrations during adolescence did not differ significantly between juvenile pair males and juvenile or mixed-age group males, but adult courtship was significantly lower in juvenile pair males compared to juvenile group males. Moreover, adult aggression was significantly lower in juvenile pair males compared to males from juvenile and mixed-age groups. Therefore, there is no evidence that differences in T during adolescence affected adult behaviour. Instead, other hormones or social learning during adolescence may be more important for shaping adult behaviour during this period (Bottjer & Johnson 1997, Schlinger 1997, Freeberg 2000).

In addition to organisational effects of T, long-lasting effects of the early social environment are often explained by effects on CORT levels (Spencer & Verhulst 2007, Banerjee et al. 2012). Zebra finches are highly social birds and social isolation affects CORT levels (Banerjee & Adkins-Regan 2011, Perez et al. 2012). Social isolation of zebra finch males during adolescence also results in delayed plumage maturation (Leader & Nottebohm 2006) and song development (Morrison & Nottebohm 1993, Jones et al. 1996) compared to socially reared controls, which could be due to elevated CORT levels caused by social stress. However, we found no differences in CORT profiles between males in the different housing conditions, suggesting there were neither differences in social stress nor an effect of CORT on the behavioural differences we found. This is in line with an earlier study that found no effect of social density on CORT levels during development (Poot et al. 2012). The decrease of CORT during early adolescence found in all social conditions in the present study could be attributed to an initial stress response when removed from the familiar environment of the natal aviaries and subsequent habituation to the new social environments. Habituation to new environments has been shown to occur within 30 min in zebra finches (Banerjee & Adkins-Regan 2011), but it is not known whether the same is true for a novel social environment. Since CORT levels in our study were high when first measured several days after introduction to the social treatments, this may reflect a stress response when adjusting to the new social environment or a maturational change of CORT profiles during development (Poot et al. 2012), although other studies suggest that basal CORT levels do not vary with age in zebra finches (Brown & Spencer 2013).

Comparison with earlier experiments

In contrast to our expectations and earlier results (Ruploh et al. 2013, 2014a), adult courtship and aggression was elevated in males reared in groups compared to males reared in pairs, although this was only statistically significant for courtship in males from juvenile groups and for aggression when males from groups were tested against males from pairs. In previous studies it was suggested that males may court and compete less at high densities to prevent costly competition (Ruploh et al. 2013). Similar behavioural differences were seen in guinea pigs and wild cavies reared under increased or instable social densities, which have been interpreted as an adaptive “queuing strategy” or “behavioural camouflage strategy” (Kaiser & Sachser 2001, Sachser et al. 2011, Siegeler et al. 2011). The results from the current study show that this interpretation has to be reevaluated for zebra finches. Interestingly, adult song rates of pair-housed males in the current study were about three times lower than in the previous studies, whereas song rates of males from juvenile groups were similar (Ruploh et al. 2013). Also, the courtship latency was higher and the level of aggressiveness shown

towards opponents was lower in males reared in pairs in the current study compared to previous studies, whereas courtship latencies and rates of aggression of males reared in groups differed less. In the following, we discuss differences between the present study and earlier experiments which may have especially affected males housed in pairs and therefore explain the discrepancy in results.

First, we kept males in rooms with constant photoperiod during adolescence, whereas previously males experienced natural variation in photoperiod. Photoperiod affects morphological, physiological and behavioural development, as well as adult behaviour in many species (e.g. Pyter & Nelson 2006, Guenther et al. 2014), including zebra finches (Zann 1996, Bentley et al. 2000, Perfito et al. 2007). Effects of the social environment might thus depend on photoperiod and thereby explain the contrasting effects between our study and the previous ones. Moreover, studies in zebra finches suggest that photoperiodic effects may be masked by stimulatory effects of food abundance and social interactions (Perfito et al. 2008, Perfito 2010). The constant photoperiod in our study might therefore have more strongly affected males housed in pairs because they experienced less social stimulation.

A second potential explanation for the differing results from previous research is a difference in acoustic stimulation during development. In this study males of different treatments were housed in the same indoor rooms, while males in previous studies were spatially farther apart and no groups containing both adults and juveniles were nearby. Since acoustic stimulation can modify adult behaviour and development (Slater et al. 1988), we suggest that males housed in pairs may have been most affected by the vocal interactions of adults and juveniles in adjacent mixed-aged groups.

Finally, male aggression was previously observed in triads (Ruploh et al. 2013) or in small groups (Ruploh et al. 2014a). If aggression of pair-reared males depends on social density, this may explain why we found low aggression when testing males in dyads. In previous studies, pair-reared males were also more aggressive than group-reared males when later tested in dyads (Ruploh et al. 2014b), but this may have been a consequence of winner-loser effects during the first tests.

Conclusion

Our study shows that the social environment during adolescence affects social interactions, plumage maturation and song development of male zebra finches with long-lasting consequences for adult courtship and aggressive behaviour. Contrary to our initial hypotheses, we do not find elevated T and CORT levels in groups as described for guinea pigs. The difference seen in T levels between males in juvenile groups and mixed-age groups may be linked to differences in song development, but not in plumage maturation or to the long-lasting effects on behaviour, and CORT is unlikely to play any role. The effect of the social environment during adolescence on other hormones should therefore be considered. The intriguing differences between our study and previous ones point to the multitude of factors shaping development. Future studies should therefore investigate how social factors interact with ecological conditions.

Chapter 3

**Flirting and fighting skills in male zebra finches
(*Taeniopygia guttata*): the role of adolescent social
experience and mating status of interaction partners**

S. Bølting, N. von Engelhardt

Manuscript

Abstract

Background Group living requires individuals to adjust their behaviour to different interaction partners across different contexts. The ability to optimise the expression of social behaviour as a function of available social information, referred to as social competence, is affected by social experience during development. During adolescence, individuals reach sexual maturity, suggesting that social experience during this life stage may be especially important for sexual behaviour, but to date, only little empirical evidence exists. Previously, we found that in male zebra finches (*Taeniopygia guttata*) adolescent rearing either in juvenile pairs, juvenile groups and mixed-age groups modifies the frequency of adult courtship singing and/or aggressiveness in sexual contexts. Differences in aggression, however, were strongly dependent on the social background of an opponent. Here, we investigated whether males differ in their adjustment of courtship and aggressive behaviour depending upon an important determinant for the success of courtship attempts, the mating status of interaction partners.

Results All males sang more towards unpaired females than towards those with a mate, and males did not differ in discrimination of females during courtship singing. However, males reared in juvenile groups sang most of all experimental males directly after being exposed to females (0 h). In addition, they reduced courtship singing towards unpaired females significantly more over 24 h compared to males reared in juvenile pairs. The level of aggressiveness shown was highest towards male opponents for all experimental males. Yet, males from both groups (0 h) or from juvenile groups (24 h), respectively, discriminated significantly more between male and female interaction partners than males from pairs. The level of aggressiveness received was low in general, but all males received most aggression from paired females (0 h).

Conclusion Male zebra finches reared in different social environments during adolescence do not differ in their ability to assess the suitability of females as potential mates. However, the social background of males influences how they compete with opponents and how they adjust over time to an environment offering mating opportunities. The results suggest that social experience during adolescence impacts on behavioural performance of males in sexual contexts, with possible consequence for their fitness.

Background

Individuals living in social groups frequently interact across a variety of contexts (e.g. feeding, mate choice, territory defence) with conspecifics that differ considerably in rank, size and internal (e.g. emotional) state. In order to respond to social challenges in an adequate way that reduces the costs and maximizes the benefits of interactions, group members rely on cognitive abilities to process and integrate social information (Shettleworth 1998). The ability to optimise the expression of social behaviour as a function of available social information is referred to as social competence (Oliveira 2009, Taborsky & Oliveira 2012).

Group members often exhibit huge inter-individual variation in social traits. So far, only few studies investigated to what extent individual variation in social competence is caused by genetic predisposition, e.g. in humans by means of twin studies (McGuire et al. 1999, Kuo et al. 2004). In contrast, many studies strongly indicate that an individual's social experience is an important factor that influences how it evaluates and chooses an appropriate behavioural response to a given stimulus. Individuals gain social experience through their own social interactions (e.g. "winner-loser effect") (Hsu et al. 2006, Rutte et al. 2006) or by observing social interactions between conspecifics ("bystander effect") (Oliveira et al. 1998, Earley 2010). In many species, for example lab mice (*CD1 (ICR)*) (Branchi et al. 2006), guinea pigs (*Cavia aepora* f. *porcellus*) (Sachser 1998, Sachser et al. 2011), and rhesus macaques (*Macaca mulatta*) (Bastian et al. 2003), a more complex social environment leads to improved social performance, which presumably leads to enhanced fitness. It has been proposed that improved social performance is due to increased opportunities for effective social learning in a more complex environment (White et al. 2010). Thereby, social experience gained during early ontogeny appears to affect the development of social competence particularly strongly. This may be attributed to the occurrence of specific sensitive windows for learning during early life (e.g. Fawcett & Frankenhuis 2015).

Given that the impact on the fitness of an individual is the ultimate measure to evaluate whether social behaviour is adaptive, many studies investigating effects of the social environment on social competence focused on sexual behaviour. In a monogamous bird species, the brown-headed cowbird (*Molothrus ater*), the social environment had profound effects on males' courtship and aggressive behaviour when introduced into a new environment with unfamiliar females. Males kept in dynamic flocks used aggression to increase dominance rank and courtship song to form associations with particular females, while males kept in stable flocks addressed courtship and aggression less selectively. As a consequence, dynamic-flock males had greater mating success than stable-flock males (White et al. 2010, Gersick et al. 2012). The experimental set up of this study mimicked the social structure of cowbirds in the wild. However, it remains open whether males from stable flocks and males from dynamic flocks differ in their ability to adjust their behaviour to the

state of conspecifics, which is a crucial requirement for group living (Taborsky & Oliveira 2012). So far, very few studies have investigated the influence of the early social environment on adult behaviour in relation to interaction partners' characteristics (but see Arnold & Taborsky 2010).

The existence of fine-tuned adjustment of behaviour has been indicated in our own study species, the zebra finch (*Taeniopygia guttata*). In zebra finches, the social environment experienced during early development has multiple effects on adult social behaviour, which has been extensively investigated for sexual behaviour, such as partner preference (Sonnemann & Sjölander 1977, Immelmann et al. 1991, Mansukhani et al. 1996, Adkins-Regan & Krakauer 2000, Holveck & Riebel 2009). Social experience gained during adolescence has also been shown to significantly influence adult sexual behaviour of zebra finches, for example courtship towards females or aggression towards rivals (Morrison & Nottebohm 1993, Jones et al. 1996, Mariette et al. 2013, Ruploh et al. 2013, Bølting & von Engelhardt 2017 (chapter 2)). This seems likely, as, during adolescence, zebra finches attain sexual maturity and adult courtship displays such as song and nest building behaviour crystallize to their final form (Zann 1996). Recently, we have shown that in competition over a single unfamiliar female, zebra finch males reared in juvenile groups (three juvenile males and three juvenile females) or in mixed-age groups (three juvenile males, three juvenile females, two adult males and two adult females) during adolescence adjust their level of aggression to the social background and hence potentially to the fighting skills of their opponent. Males reared in juvenile pairs (one juvenile male and one juvenile female) did not show such a behavioural adjustment (Bølting & von Engelhardt 2017 (chapter 2)). This finding strongly points to differences in courtship competence between males reared in juvenile pairs, juvenile groups or mixed-age groups. More precisely, it seems likely that males differ in their ability to evaluate and adequately respond to behavioural displays of interaction partners in sexual contexts, probably due to differences in social learning opportunities during development. In an enriched social environment, an increased ability to behaviourally adjust to various social stimuli is likely to be essential for survival and reproduction.

To verify this assumption, it is important to investigate whether males also differentially adjust the frequency of courtship singing to variation in behavioural displays of females. In addition, testing males' aggressive behaviour in a different context might provide more information about their ability to adjust fighting to the state of rivals. Until now, only differences in the total frequency of courtship singing towards a single unfamiliar female between males from different social environments during adolescence have been described (Bølting & von Engelhardt 2017 (chapter 2)).

Behavioural displays in monogamous species, such as zebra finches, vary remarkably between females and males depending upon their mating status, i.e. whether they have developed a pair bond with an individual of the other sex or not. Males without a mate (“unpaired”) compete for access to females, while males with a mate (“paired”) aggressively attack rivals due to increased competition over resources, such as food or nesting sites (Zann 1996). Costly physical conflicts with males, as well as their female mates, can be elicited by courting a paired female (Silcox & Evans 1982, Zann 1996). Alternatively, courting a paired female may just waste energy, as paired females may ignore courtship attempts of males other than their mate (Zann 1996). Rejection and solicitation of courtship attempts of males is characteristically shown by paired and unpaired females, respectively, even though extra-pair copulations with a paired female do occur in zebra finches (Birkhead et al. 1990, Houtmann 1992, Burley et al. 1996, Zann 1996, Forstmeier 2007, Griffith et al. 2010). Hence, the mating status of interaction partners can be important, both for physical health and reproductive success. Consequently, males should behave cautiously towards paired rivals and only benefit from displaying a high level of aggression when their own competitiveness is higher than that of their interaction partner. Furthermore, males should benefit from directing more courtship song towards unpaired females than towards paired females.

The aim of the present study was to understand whether the social environment during adolescence influences how adult zebra finch males adjust their courtship and aggressive behaviour to interaction partners differing in mating status. Therefore, we investigated whether males reared in juvenile pairs, juvenile groups or mixed-age groups differ in the use of courtship and aggression when introduced into a flock of two established breeding pairs and two additional females without a mate. We predicted that males reared in groups during adolescence would, compared to males reared in pairs, show an increased difference in courtship singing towards paired and unpaired females and an increased difference in aggression towards males and females. We suggest that this would be due to an increased ability to discriminate the mating status of females, and an increased ability to fight against same-sex rivals. As a consequence of differences in behavioural selectivity, we predicted that group-reared males would receive less aggression from interaction partners than pair-reared males. We further predicted that the behavioural performance of males from juvenile groups and mixed-age groups might also differ, due to differences in learning opportunities provided by same-aged and older conspecifics during development.

Methods

Subjects and housing conditions

The experimental subjects were male domesticated zebra finches (*Taeniopygia guttata*). Birds were bred at the University of Bielefeld under a controlled 14:10 h light: dark cycle (lights on at 7:00 am), but with additional daylight entering through windows. At the beginning of the adolescent period (average age 41 days, range: 36-45), males were moved to small aviaries (200 cm high x 100 cm wide x 100 cm deep) into groups differing in social composition and received no natural daylight anymore. Housing rooms were lighted by fluorescent full-spectrum light tubes (Osram, Biolux, L58W/965). Males were kept in juvenile pairs (one juvenile male and one juvenile female), in juvenile groups (three juvenile males and three juvenile females), or in mixed-age groups (three juvenile males, three juvenile females, two adult males and two adult females). Adults assigned to the mixed-age groups were unfamiliar mated pairs. Siblings were never put in the same group and allocation over the three social treatments was randomized. After the end of the adolescent period (average age 110 days, range: 104-114), males were transferred from the treatment aviaries into single cages (30 cm high x 40 cm wide x 40 cm deep) in the same room where they had hatched. The start and end point for the social treatments during adolescence as well as the single housing were chosen according to previous experiments (Ruploh et al. 2013, Bischof et al. 2002) to ensure comparability between studies. Single housing after the social treatment period also ensured that any long-term behavioural modifications could exclusively arise through differences in the social environment experienced during adolescence. Experimental treatment groups during adolescence and individually housed adults were visually but not acoustically isolated from each other. Birds had *ad libitum* access to seed food and water throughout, and this diet was regularly supplemented by germinating seeds, fresh greens and a water bath.

Experimental events during adolescence and in early adulthood

During the social treatment, males experienced several experimental procedures. In brief, males were observed three times for the occurrence of the behavioural patterns *plastic song*, *song* and *social interactions* using focal animal sampling and continuous recording (Martin & Bateson 1993). To analyse testosterone and corticosterone levels during development, blood samples were collected three times from the ulnar vein of males. In addition, males were visually scored once a week for the development of the adult male colouration, considering the beak, cheek patches and breast stripes. Body mass measurements were conducted five times during adolescence to analyse the weight gain of males. In early adulthood, after some

time of individual housing, female directed courtship singing of focal males (average age: 158 days, range: 152-161) was quantified by presenting an unfamiliar female in a cage attached to the front of a male's cage. Furthermore, male directed aggressive behaviour of focal males was measured (mean age: 165 days, range 161-169) in dyads by placing two males together in a cage with an unfamiliar female. Opponents of focal males in the aggressiveness test were males reared in one of the other two social treatments during adolescence (for further details see Bølting & von Engelhardt 2017 (chapter 2)).

Social competence test

At an average age of 328 days (range: 283-371), courtship and aggressive behaviour towards interaction partners differing in mating status was recorded for 41 males (10 males from juvenile pairs, 16 males from juvenile groups and 15 males from mixed-age groups). For this purpose, males were singly introduced into one of four small flocks of unfamiliar conspecifics in a small aviary (200 cm high x 100 cm wide x 100 cm deep) for 24 hours. Each flock contained two established breeding pairs ("paired males" and "paired females") and two females without a mate ("unpaired females"). Birds had been housed in these flocks for twelve days before the start of the tests. They were symmetrically ringed before forming the flocks using two black rings and two identical, individually unique colour rings to ensure individual identification during observations. Focal males were ringed a day before their introduction into the flocks. Assignment of colour rings was randomized within sex and mating categories, and balanced across social treatments. Birds in different flocks could hear, but not see each other. Light conditions, food and water provision during the social competence test were the same as during the social treatment phase.

Before the start of the test and immediately after its end, males were weighed to the nearest 0.1 g to assess body mass changes during the test period.

Behavioural observations of focal males were conducted twice for 30 minutes – once directly after their introduction into a flock ("0 h") and once 24 hours later ("24 h"). In the first 15 minutes of each observation, the number of directed song motifs produced was counted and the identity of the interaction partner was recorded. As courtship song was only once directed towards a male, only female directed song was included in the analyses. If a male did not sing, it was assigned a motif number of zero. This happened only in the observations after 24 h, but there for the majority of males (only three males from juvenile pairs, two males from juvenile groups and three males from mixed-age groups still sang after 24 h). In the second half of each observation, the males' aggressive behaviour was recorded for 15 minutes by counting the number of chases directed towards another individual or directed to the focal male from another individual. The identity of the interaction partner for aggressiveness was recorded as well.

Behavioural observations were conducted using focal animal sampling and continuous recording (Martin & Bateson 1993). Observations were always performed between 08.00 am and 12.00 pm, as four males were tested consecutively per day, each in a different flock. Assignment of focal males to the different flocks was balanced across social treatments. After the removal of a focal male from a flock, the next focal male was introduced at the earliest 48 h hours later.

The breeding pairs used for the social competence test were birds that had already successfully bred with each other for at least two consecutive broods. The unpaired females have not had access to a male partner for at least one year. Although zebra finches are monogamous birds, re-pairing can occur, for example after death of a partner and this happens quite often as in nature mortality is high in zebra finches (Zann 1996). Furthermore, extra-pair copulations do occur in zebra finches (Zann 1996). Therefore, the actual pairing status of breeding pairs was checked every morning for 5 minutes before behavioural observations of focal males. If pair-bonding behaviour, such as preening, following, courtship singing or copulation (Zann 1996), was observed during this period between a paired stimulus male and a previously unpaired female, the focal male tested in this flock was excluded from the study. This was done because the pairing status of birds was crucial for the interpretation of the results from the social competence test. In total, three males (one from juvenile pairs and two from juvenile groups) had to be excluded.

The final sample size for the social competence test was therefore 38 males (9 males from juvenile pairs, 14 males from juvenile groups and 15 males from mixed-age groups).

Data analysis

Some data had to be transformed to achieve equal variances between social treatment groups and normally distributed residuals. Variances and distributions were assessed visually using variance plots, histograms of residuals and Q-Q plots. For some data, different transformations were tested, but all only resulted in an approximation of equal variances and normally distributed residuals. However, significances of results did not change with transformation method applied and, therefore, the transformations that resulted in the most equal distribution of variances and residuals were chosen. The courtship song motif rate (n/min) and the rate of chases (n/min) were analysed after a square root transformation. Body mass data, analysed as percentage of initial body mass lost, met the criteria of variance homogeneity and normally distributed residuals without transformation and were thus analysed without transformation. All data were analysed by linear mixed models (LMM), assuming a normal distribution.

Data were analysed in R 3.1.2 by mixed effects models using a maximum likelihood approach (package lme4). Significances of effects were calculated using likelihood ratio tests.

The analyses included a random effect of the flock males were introduced to ("stimulus group ID"), a random effect of the experimental aviary during adolescence ("treatment group ID"), a random effect of the male's family ("nest ID"), and a random effect of the male's identity ("male ID") for data with multiple measures for each male. "Nest ID" and "male ID" were kept in all models to control for non-independence of multiple measures from the same male and from brothers allocated to different treatment groups. A random effect of "stimulus group ID" and "treatment group ID" was removed from the models, if it was not statistically significant ($p > 0.1$). Differences in body mass loss between the first and the second observation between the three social treatments were analysed with treatment ("social treatment") as the only fixed effect.

Differences in courtship motif rate and rate of chases towards / from different interaction partners between the three social treatments were analysed for each observation separately. In these analyses, the effects of treatment ("social treatment") and interaction partner ("interaction partner") and their interaction was tested. Whenever an interaction of "social treatment" and "interaction partner" was not statistically significant ($p > 0.1$), it was removed from the model. Subsequently, the main effects of "social treatment" and "interaction partner" were tested. Even when not significant, the main effects were always kept in the final models. Whenever there was a significant interaction effect of "social treatment" and "interaction partner" or a significant main effect of "social treatment" in the final model, subsequent pairwise comparisons between two experimental groups were conducted. If there was no longer an interaction effect when comparing two experimental groups, a main effect of "social treatment" was also tested. Post-hoc tests were conducted using Sidak adjustments to account for multiple testing.

Differences in courtship motif rate and rate of chases between the first and the second observation between the three social treatments were analysed for each interaction partner separately. In these analyses, the effects of treatment ("social treatment") and observation ("observation") and their interaction was tested. Whenever an interaction of "social treatment" and "observation" was not statistically significant ($p > 0.1$), it was removed from the model. Subsequently, only the main effect of "observation" was tested in order to identify whether behavioural patterns changed over time. Even when not significant, the main effect of observation was always kept in the final models, together with the main effect of "social treatment". Only when there was a significant interaction effect of "social treatment" and "observation" in the final model, subsequent pairwise comparisons between two experimental groups were conducted. If there was no longer an interaction effect when comparing two

experimental groups, a main effect of “observation” was not tested. Post-hoc tests were conducted using Sidak adjustments to account for multiple testing.

Graphs show the means \pm standard error (SE) estimated with “social treatment” or with “social treatment” and “interaction partner”, including the random effects. The significance level α was set at $p < 0.05$.

Results

Body mass loss

Males from different social treatments did not differ significantly in the percentage of initial body mass lost during the time in the new flock (LMM: social treatment: $\chi^2 = 0.35$, $df = 2$, $p > 0.1$).

Courtship song motif rate

There was no significant effect of the interaction between social treatment and interaction partner on the motif rate of males in the first observation (0 h; LMM: social treatment \times interaction partner: $\chi^2 = 3.16$, $df = 2$, $p = 0.21$; Fig. 1A). There was a significant main effect of social treatment (LMM: social treatment: $\chi^2 = 10.52$, $df = 2$, $p < 0.01$). Post-hoc tests revealed that males from juvenile groups sang significantly more towards both paired and unpaired females than males from juvenile pairs or mixed-age groups (LMM: social treatment: $\chi^2 > 5.96$, $df = 1$, $p < 0.05$), while the latter two treatments did not differ

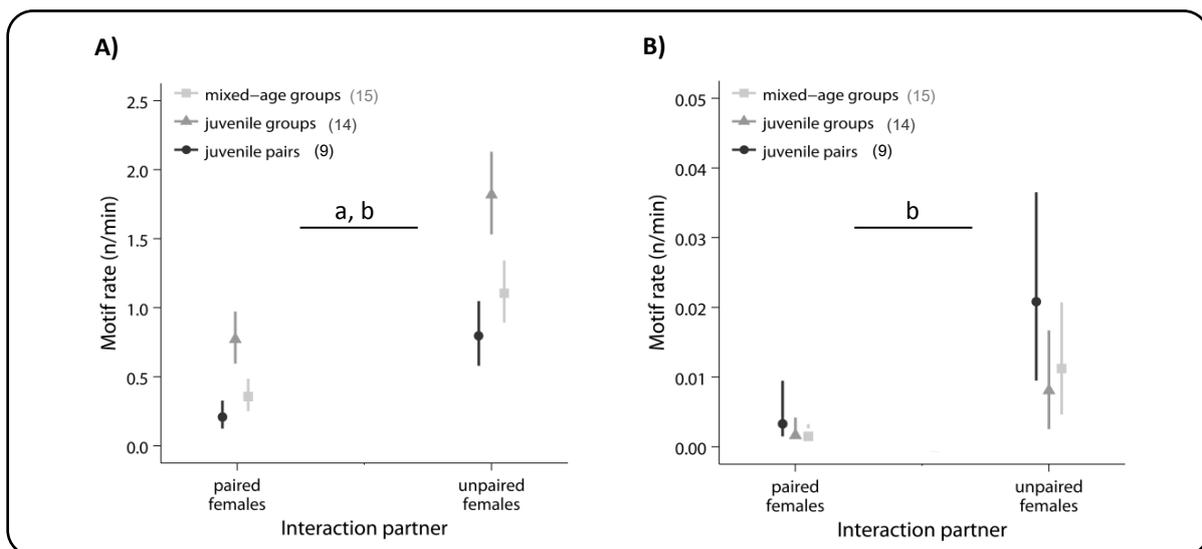


Fig. 1: Courtship song motif rate. A) In the first observation, the motif rate of courtship song was significantly influenced by both social treatment (a) and interaction partner (b). **B)** In the second observation, the motif rate was only significantly influenced by interaction partner (b). For details, see text. Shown are means \pm SE and sample sizes in brackets. Y-axes scales of A) and B) differ by a factor of 50.

significantly from each other (LMM: social treatment: $\chi^2 = 1.50$, $df = 1$, $p = 0.22$). A significant main effect of interaction partner (LMM: interaction partner: $\chi^2 = 19.75$, $df = 1$, $p < 0.001$) indicated that all males sang significantly more towards unpaired females than towards those with a mate.

In the second observation, the males' motif rate of courtship singing was neither significantly influenced by an interaction between social treatment and interaction partner (24 h; LMM: social treatment x interaction partner: $\chi^2 = 1.67$, $df = 2$, $p = 0.43$; Fig. 1B) nor by social treatment (LMM: social treatment: $\chi^2 = 1.15$, $df = 2$, $p = 0.56$). However, males of all treatments sang more towards unpaired females than towards those with a mate, as shown by a significant main effect of interaction partner (LMM: interaction partner: $\chi^2 = 7.09$, $df = 1$, $p < 0.01$).

All males reduced courtship singing towards paired females from the first to the second observation to a similar degree (LMM: social treatment x observation: $\chi^2 = 2.15$, $df = 2$, $p > 0.1$; observation: $\chi^2 = 57.28$, $df = 1$, $p < 0.001$). The reduction in courtship singing towards unpaired females differed significantly between males from different social treatments (LMM: social treatment x observation: $\chi^2 = 12.30$, $df = 2$, $p < 0.01$). Males from juvenile groups reduced courtship singing towards unpaired females significantly more than males from juvenile pairs (LMM: social treatment x observation: $\chi^2 = 8.80$, $df = 2$, $p < 0.01$), while there was no significant difference in the reduction of courtship singing towards unpaired females between the other treatments (LMM: social treatment x observation: $\chi^2 < 1.07$, $df = 1$, $p > 0.1$).

Aggression directed towards other birds

The rate of chases initiated by focal males in the first observation was significantly influenced by an interaction of social treatment and interaction partner (0 h; LMM: social treatment x interaction partner: $\chi^2 = 19.94$, $df = 4$, $p < 0.001$; Fig. 2A). Comparing pairs of experimental groups revealed that group-reared males differed significantly from pair-reared males in the level of aggression shown towards different interaction partners (LMM: social treatment x interaction partner: $\chi^2 > 11.9$, $df = 2$, $p < 0.01$). Males from juvenile groups and males from mixed-age groups neither differed significantly from each other in the level of aggression shown towards different interaction partners (LMM: social treatment x interaction partner: $\chi^2 = 1.26$, $df = 2$, $p > 0.1$), nor in the mean level of aggression shown (LMM: social treatment: $\chi^2 = 0.43$, $df = 1$, $p > 0.1$). Group-reared males showed much more aggression towards males than towards either type of females, while the difference in the level of aggression shown towards males and females was marginal for pair-reared males.

The rate of chases initiated by focal males in the second observation strongly tended to be influenced by an interaction of social treatment and interaction partner (24 h; LMM: social

treatment x interaction partner: $\chi^2 = 9.46$, $df = 4$, $p < 0.051$; Fig. 2B). This time, the difference in aggression towards different interaction partners (most aggression towards males) was significantly larger for males from juvenile groups than for males from juvenile pairs (LMM: social treatment x interaction partner: $\chi^2 = 9.12$, $df = 2$, $p = 0.03$). There was no significant difference in the rate of chases shown towards different interaction partners between the other treatments (LMM: social treatment x interaction partner: $\chi^2 < 3.96$, $df = 2$, $p > 0.1$). Furthermore, there was no significant difference between males from juvenile pairs and males from mixed-age groups or between males from juvenile groups and males from mixed-age groups in the mean level of aggression shown (LMM: social treatment: $\chi^2 < 0.25$, $df = 2$, $p > 0.1$).

All males reduced the rate of chases towards males and unpaired females between the first and the second observation to a similar degree (LMM: social treatment x observation: $\chi^2 < 2.63$, $df = 2$, $p > 0.1$; observation: $\chi^2 < 14.77$, $df = 1$, $p < 0.02$). There was no significant change in the rate of chases towards paired females between the first and the second observation for any of the males (LMM: social treatment x observation: $\chi^2 = 1.39$, $df = 2$, $p > 0.1$; observation: $\chi^2 < 1.81$, $df = 1$, $p > 0.1$).

Aggression directed towards focal males

There was no significant effect of the interaction between social treatment and interaction partner on how often focal males were chased in the first observation (0 h; LMM: social treatment x interaction partner: $\chi^2 = 4.95$, $df = 4$, $p > 0.1$; Fig. 3A). There was also no main effect of social treatment on the rate of chases directed towards focal males by stimulus birds (LMM: social treatment: $\chi^2 = 0.12$, $df = 2$, $p > 0.1$), but there was a significant main effect of interaction partner (LMM: interaction partner: $\chi^2 = 14.93$, $df = 2$, $p < 0.01$). Males received most chases from paired females, less chases from unpaired females and least chases from males.

After 24 hours in the new flock, there was no significant effect of the interaction between social treatment and interaction partner on the rate of chases directed towards focal males (24 h; LMM: social treatment x interaction partner: $\chi^2 = 3.90$, $df = 4$, $p > 0.1$; Fig. 3B). Furthermore, there was neither a significant main effect of social treatment (LMM: social treatment: $\chi^2 = 0.78$, $df = 2$, $p > 0.1$) nor of interaction partner (LMM: interaction partner: $\chi^2 = 4.30$, $df = 2$, $p > 0.1$). All focal males were chased similarly often by males, paired females and unpaired females.

Overall, the rate of chases directed towards focal males by paired females significantly decreased between the first and the second observation, but was not significantly influenced by social treatment (LMM: social treatment x observation: $\chi^2 = 0.88$, $df = 2$, $p > 0.1$; observation: $\chi^2 = 10.88$, $df = 1$, $p < 0.001$). The rate of chases directed towards focal males

by males and unpaired females did not change significantly between the first and the second observation (LMM: social treatment x observation: $\chi^2 < 1.04$, $df = 2$, $p > 0.1$; observation: $\chi^2 < 1.67$, $df = 1$, $p > 0.1$).

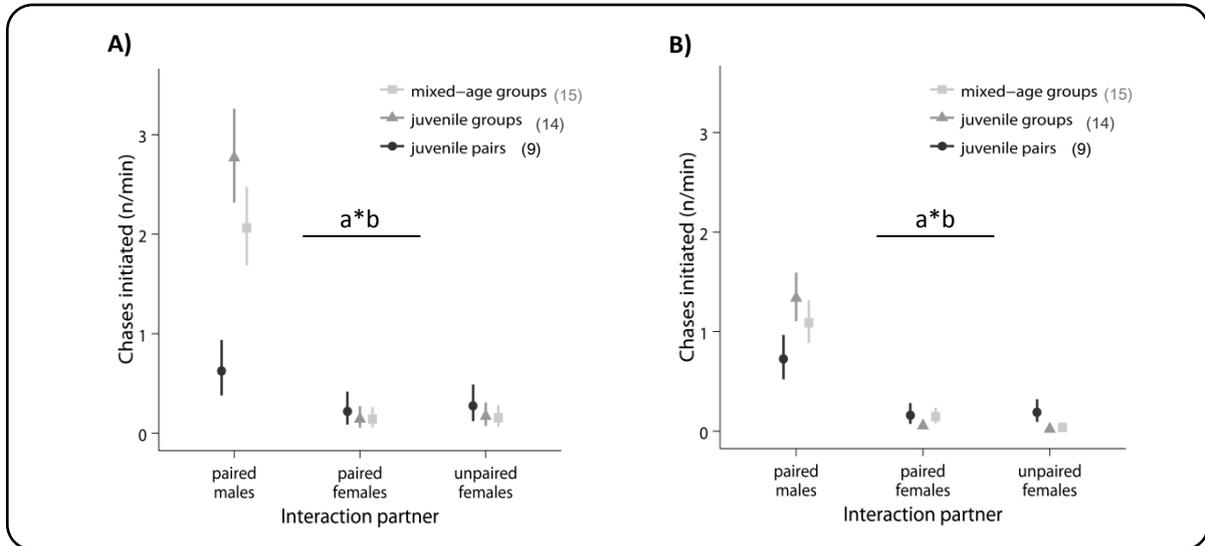


Fig. 2: Aggression directed towards other individuals. **A)** In the first and **B)** in the second observation the rate of chases initiated by the focal males was significantly influenced by an interaction of social treatment and interaction partner (a*b). For details, see text. Shown are means \pm SE and sample sizes in brackets.

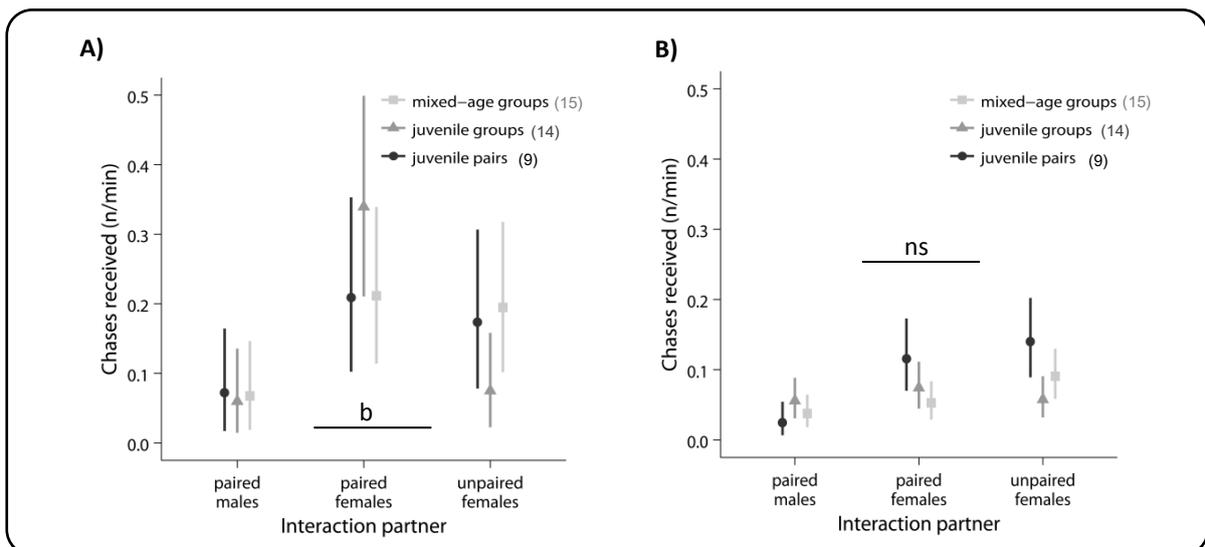


Fig. 3: Aggression directed towards focal males. **A)** In the first observation, the rate of chases received was significantly affected by interaction partner (b) for all males. **B)** In the second observation, there was no significant effect (ns) of social treatment or of interaction partner on the rate of chases males received. For details, see text. Shown are means \pm SE and sample sizes in brackets.

Discussion

Regardless of social experience during adolescence, male zebra finches were able to assess the suitability of females in their environment as potential partners: All males sang more towards unpaired females than towards those with a mate. The discrimination of females according to their mating status was shown directly after the introduction into the new flock as well as 24 h later. Contrary to what we expected, however, males from different social rearing environments during adolescence showed no difference in discrimination of paired and unpaired females during courtship singing.

The display of aggression of experimental males towards different interaction partners was significantly influenced by the social environment during adolescence, as expected. Directly after the introduction into the new flock, group-reared males exhibited an increased discrimination of males and females for aggressive interactions compared to paired-reared males, and this difference persisted for 24 h between males from juvenile groups and males from juvenile pairs.

The level of aggression directed towards experimental males by stimulus birds was not affected by the social environment during adolescence, and was in general relatively low. All males received most aggression from paired females at 0 h.

Courtship performance

Our findings suggest that in zebra finches, the ability to identify available mates in the environment may either be required already before adolescence or develops similarly during adolescence under all social conditions experienced by the experimental males.

In a previous study on zebra finches, another critical component of mate choice has been shown to be influenced by social experience before independence, namely whether individuals in adulthood choose partners of the opposite sex (Adkins-Regan & Krakauer 2000). Early social experience can even modify sexual preferences beyond species borders in these birds, as males raised by Bengalese finches (*Lonchura striata*) choose females of the foster species instead of their own species as mates when tested in a double-choice test in early adulthood (Immelmann et al. 1991).

Notably, information about partner preferences acquired during early development may need to be validated by first courtship experience (Immelmann et al. 1991, Bischof & Clayton 1991). In case of our birds, pair-reared males could not compare between behavioural responses of different females to their first own courtship attempts during adolescence. However, responses of a single female during adolescence may have been sufficient to learn to distinguish between rejection and solicitation and hence may have facilitated the ability of males to discriminate between females of different mating status (Zann 1996). The significance of experience with a single female during adolescence has also been revealed in

a previous study (Mariette et al. 2013). In that study, zebra finch males that failed to pair with a single female in late adolescence had a lower pairing success in adulthood than males that did not fail to pair with a female (Mariette et al. 2013). In order to better understand the role of courtship experience during adolescence, a control group of males housed individually during adolescence should be set up in a future experiment.

In general, a very early acquisition of the ability to discriminate between females of different mating status may be explained by its fundamental importance to successfully reproduce once sexual maturity is attained. Zebra finches are monogamous and typically form lifelong pair bonds in which males and females guard their mates against approaches by same-sex rivals (Zann 1996). Nonetheless, extra-pair copulations do occur in zebra finches (Houtmann 1992, Zann 1996, Forstmeier 2007) and the rate of extra-pair fertilizations seems much higher in captive populations (28 %) (Burley et al. 1996) than in free-living birds (1.7 % - 2.4 %) (Birkhead et al. 1990, Griffith et al. 2010). However, trying to obtain extra-pair fertilizations might be costly for males, as it might increase the risk to lose paternity in their own nests to competitors (Burley et al. 1996). In addition, courting already paired females often provokes physical conflicts with the females and their mates (Silcox & Evans 1982, Zann 1996), which bears the risk of injuries. Instead, when courting females that are not paired to another male, the chance of pair formation and reproductive success is higher. Hence the ability to discriminate between paired and unpaired females and to direct courtship singing accordingly should be beneficial for males. Similarly, it has been suggested that male zebra finches benefit from being able to assess the fertility status of females (Burley et al. 1996).

The fact that in the present study all experimental males received very little aggression from all interaction partners throughout the experiment confirms that males directed their courtship singing appropriately towards different interaction partners. Especially the very low level of aggression received from paired males may indicate that these were not stimulated to behave aggressively by the courtship singing of experimental males towards their own mates. Alternatively, paired males might have shown little aggression towards experimental males because paired males were inferior to experimental males and intimidated by their fighting skills (see “*Use of aggression*”). Noticeably, experimental males received the highest level of aggression from paired females. On closer examination, though, it seems that this high level of aggression was mainly based on chases directed towards males from juvenile groups (see Fig. 3). This may be attributable to differences in the total frequency of courtship singing between experimental males.

Males reared in juvenile groups sang significantly more than males reared in juvenile pairs and mixed-age groups. The increased level of singing may have resulted in an increased level of aggression of paired females against males from juvenile groups. This idea is

supported by the fact that a significant reduction of the males' courtship singing between the first and the second observation was accompanied by a significant reduction of aggressiveness shown by paired females. In contrast to paired females, unpaired females might have been more receptive towards the increased level of courtship singing from males from juvenile groups because they were still choosing a mate. It seems that they directed less chases towards those than towards males from the other treatments. However, the difference between experimental males in the level of aggression received from paired and unpaired females was statistically not significant. It might be that the temporal separation of the behavioural recordings of courtship and aggression in the present study distorted how males' courtship affected behavioural responses of females. To allow a more precise statement about the courtship competence of males, simultaneous recording of courtship and aggression is needed.

Experimental males might have differed in how stressful they perceived the introduction into the new flock, hence resulting in differences in the total frequency of courtship singing. We did not find a significant difference in weight loss between males, which is often used as an indicator for stress in vertebrates (e.g. Tamashiro et al. 2005, Sachser et al. 2011). However, males may differ in stress hormone levels, e.g. corticosterone, which can be influenced by the early social environment in zebra finches (Banerjee et al. 2012). Corticosterone has been shown to affect courtship in zebra finches (Spencer et al. 2003). As we did not measure hormone levels in the present study, we can neither verify nor exclude this option. The finding that males from juvenile groups sang most of all experimental males strongly resembles previously detected differences in courtship singing of males (Bölting & von Engelhardt 2017 (chapter 2)). This indicates that the social environment during adolescence induces stable behavioural modifications. This has been reported before in zebra finches (Ruploh et al. 2013, 2014b) and other species (Sachser et al. 2013) and highlights the importance of adolescence as a sensitive period in an individual's life (Fawcett & Frankenhuis 2015). Nonetheless, zebra finch males have been found to adapt their courtship behaviour over time to the context in which they are tested (Ruploh et al. 2014a, this study).

In the present study, courtship singing was significantly higher for all males directly after the introduction into the unfamiliar flock than 24 h later. This change in singing activity may especially be due to an initially increased sexual motivation of males after sexual deprivation during individual housing (Pröve 1987). Furthermore, courtship singing is often used by zebra finch males at the beginning of sexual encounters with females, but decreases in relevance over time. It is replaced by other behavioural patterns, such as allopreening or sitting in contact, once a pair bond is established (Zann 1996) and might therefore cease because it no longer has an effect. However, the decrease in courtship singing towards females partly differed between males from different social rearing environments. All males

reduced courtship singing towards paired females similarly, but not towards unpaired females. Towards the latter, males reared in juvenile groups reduced courtship singing significantly more than males reared in juvenile pairs. This suggests that males reared in juvenile groups adjusted their behaviour more during the 24 h in the new social environment than males from juvenile pairs. It has been previously proposed that males reared in groups and males reared in pairs might differ in their ability to behaviourally adjust to social contexts (Ruploh et al. 2014a). We cannot say with certainty whether the difference in the decrease of courtship singing we found was due to a more pronounced decrease in sexual motivation of males from juvenile groups or an increased success in pair bonding with unpaired females. However, their higher behavioural flexibility might indicate that males from juvenile groups exhibit a higher courtship competence than males from juvenile pairs. Behavioural flexibility is a necessary, though not sufficient, prerequisite for social competence (Taborsky & Oliveira 2012). To call it social competence, the behavioural adaptation must come along with increased fitness correlates (Taborsky & Oliveira 2012). Fitness parameters of experimental males should therefore be investigated in future studies.

Use of aggression

The aggressive behaviour shown by experimental males strongly indicates that the social environment during adolescence influences how zebra finch males compete with same-sex opponents in adulthood. This supports previous findings that the aggressiveness of zebra finch males in a competitive context is affected by the social environment during adolescence (Bölting & von Engelhardt 2017 (chapter 2)).

In the presence of a single unfamiliar female, differences in the level of aggression displayed by experimental males depended on the social background of the opponent. Males reared in juvenile groups or mixed-age groups showed an increased level of aggression when tested against an opponent from a juvenile pair, but a low level of aggression when tested against an opponent from the other group treatment. In contrast, males from juvenile pairs showed a low level of aggression towards males from both juvenile groups and mixed-age groups (Bölting & von Engelhardt 2017 (chapter 2)). We argued that the social rearing environment of a group offered increased opportunities for males to improve and learn about their own fighting skills in relation to those of others. Unlike group-reared males, pair-reared males had no opportunity to learn how to display appropriate social behaviour towards same-sex conspecifics and hence may have triggered aggression in group-reared males (Bölting & von Engelhardt 2017 (chapter 2)).

Even though some behavioural patterns of aggression may have been acquired by males already before adolescence, e.g. through early play behaviour (e.g. Meaney & Stewart 1981, but see Pellis & Pellis 1988), the lack of opportunities to validate these behavioural patterns

later in a sexual context (as e.g. found for partner preference, see Immelmann et al. 1991, Bischof & Clayton 1991) may have characterized pair-reared males as inferior opponents in a competition for access to a female.

In the present study, opponents of experimental males were paired males. In these, the motivation to display aggression towards male conspecifics is often increased. Paired males have to defend their mates against approaches by same-sex rivals and intensively compete over resources such as food and nesting sites (Zann 1996) in order to increase their reproductive success. There were no nest boxes in our test setting, but individuals of both sexes were observed to aggressively defend food dispensers, into which females occasionally even laid eggs. Hence, there probably was competition between individuals in the present study. It was therefore unexpected that group-reared males again exhibited a significantly higher level of aggression compared to pair-reared males, because we expected that experimental males should have behaved rather cautiously towards paired males in order to avoid being attacked.

However, paired males in the present study showed a very low level of aggression towards experimental males and did not differentially address aggression towards experimental males from different social rearing environments. This might indicate that paired males were for some other reason less skilled in fighting than experimental males and unable to assess potential differences in competitiveness of pair-reared and group-reared individuals. Paired males were kept in uni-sex aviaries after sexual maturity until they were paired with females. Hence, they never experienced any sexual competition with other males before the start of our experiment and may never have learned how to appropriately behave towards rivals.

In addition to the potential inferiority of paired males, the own previous fighting experience of experimental males in adulthood might have modulated their level of aggression. When first tested for their competitiveness, group-reared males either made the experience of being superior or at least equally skilled compared to their opponent. In contrast, pair-reared males only experienced defeat. In many species, the experience of winning in a competition affects the behaviour during future contests and increases the likelihood to again come off as a winner, while the experience of losing is likely to cause further defeat, regardless of the identity of future opponents (“winner-loser effect”) (reviewed e.g. in Rutte et al. 2006, Hsu et al. 2006).

In order to further investigate whether experimental males are able to adjust their aggressive behaviour to the state of rivals, further tests are needed, using for example males that are preselected to be highly competitive towards other males. It should be considered, though, that both the state of rivals and the context in which males are tested (high vs. low competition) may influence the aggressive behaviour of males reared under different social environments during adolescence.

The present study indicates that males from mixed-age groups are more flexible than males from juvenile groups in the adjustment of aggression over time. After 24 h in the new flock, males from mixed-age groups did not differ anymore from pair-reared males in the level of aggression shown towards different interaction partners. Only males from juvenile groups and juvenile pairs still differed in the discrimination of interaction partners. Hence, the age structure of the social environment experienced during adolescence seems to influence the social competence of zebra finch males. This has also previously been shown in cichlids (Arnold & Taborsky 2010).

Conclusion

The present study shows that the social environment during adolescence does not affect the ability of males to assess the mating status of females and to address courtship singing accordingly. However, group-rearing increases the fighting behaviour of males towards paired opponents in a competitive sexual context. In addition, group-rearing affects the adjustment of courtship and aggression of males over time. This may indicate increased sexual competence of males growing up in an enriched social environment, which ensures access to valuable resources and hence leads to enhanced fitness parameters of such individuals. Future studies should investigate whether males from different social environments during adolescence differ in fitness when competing for access to females across different contexts. Notably, despite the ability of males to behaviourally adjust to a competitive context over time, we found evidence that the social environment during adolescence induces stable behavioural modifications. This highlights the importance of the adolescent life stage as a sensitive period.

Chapter 4

Male reproductive success is influenced by the social environment during adolescence in the monogamous zebra finch (*Taeniopygia guttata*)

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Manuscript

Abstract

Background Although the social rearing environment during adolescence can strongly influence adult sexual behaviour of males, very little is known about the consequences for reproductive success. Variation in courtship and aggression is likely to affect reproductive success, especially if females can be choosy during mate choice and there is high potential for male-male competition. In order to test this idea, we investigated the effects of different social rearing conditions on different parameters of the reproductive success of male zebra finches (*Taeniopygia guttata*) in a female-restricted breeding experiment. Males were reared during adolescence in mixed-sex juvenile pairs, mixed-sex juvenile groups and mixed-sex mixed-age groups with adults.

Results The social environment during adolescence influenced males' reproductive success. More group-reared males obtained paternity than pair-reared males. In addition, the number of offspring sired and the number of nests with offspring were significantly higher for males reared in groups than for males reared in pairs. In nests in which they sired offspring, males from different group treatments did not differ in paternity success. Furthermore, the proportions of nests with exclusive and with shared paternities did not differ between males from different group treatments.

Conclusion Our results suggest that zebra finch males reared in an enriched social environment during adolescence are better adapted to a life under conditions of high mate-competition, as revealed by improved reproductive success. The high incidence of multiple paternities in group-reared males may indicate the use of an alternative reproductive strategy. An increased attractiveness and/or competitiveness may enable individuals grown up under enriched environmental conditions to accrue fitness gains through higher mating success and increased extra-pair paternities.

Background

The widespread occurrence of early developmental influences on the morphology, physiology and behaviour of individuals strongly points to an adaptive phenotypic shaping in many species of different taxa (arthropods: Krueger & Dodson 1981, fish: Arnold & Taborsky 2010; birds: Ruploh et al. 2013; mammals: Lee & Zucker 1988, Sachser et al. 2011, Guenther & Trillmich 2013). According to the Predictive Adaptive Response (PAR) hypothesis, early life environmental cues predict future prevailing conditions of an individual's habitat and induce phenotypic modifications that improve the fitness of the individual in that habitat at some point during its life (Bateson et al. 2014).

The long-lasting influence of the social environment during the prenatal and early postnatal period on adult behaviour of individuals is well known (Adkins-Regan & Krakauer 2000, Kaiser & Sachser 2005, Jarvis et al. 2006, Branchi 2009, Curley et al. 2009, Müller et al. 2016). The adolescent life stage has recently been considered as another developmental period of enhanced plasticity in which adult behaviour can be shaped (Fawcett & Frankenhuis 2015). Moreover, adolescence may provide an opportunity to correct errors in response to the environment at earlier life stages, which might be important if the environment changes and differs from that experienced earlier on. In many species, the social environment changes considerably during adolescence (Baker 1978, Zann 1996, Spear 2000). In addition, individuals in many species reach sexual maturity during adolescence and adult courtship displays crystallize to their final form (e.g. Zann 1996). Due to the potential effects on reproduction, lasting environmental effects on sexual traits during this life stage are therefore likely to be especially relevant for an individual's fitness. However, this has been verified in few species. For example, in guinea pigs (*Cavia aepera f. porcellus*), males reared in large mixed-sex colonies during adolescence exhibit little aggression towards same-sex opponents and easily integrate into an unfamiliar group of conspecifics in adulthood. In contrast, males reared in pairs are highly aggressive and suffer from severe endocrine stress responses in the same situation (Sachser et al. 2011). Under conditions of low population density, pair-reared males gain increased reproductive success compared to group-reared males (Zimmermann et al. 2017).

Both morphological and behavioural sexual traits are used by organisms to choose a mating partner. In many species, females prefer males which exhibit more colourful traits over males with less intense colouration (Brooks & Caithness 1995, West & Packer 2002, Waitt et al. 2003, Naguib & Nemitz 2007). Furthermore, males which display high levels of courtship (Bischoff et al. 1985, Collins et al. 1994, Vinnedge & Verrell 1998, Shamble et al. 2009) or aggression (Barlow 1986, Doutrelant & McGregor 2000; but see Qvarnström & Forsgren 1998) often appear to be more attractive to females than males which display only little courtship or aggression. As a result, the more attractive individuals usually have a higher

mating success than the less attractive individuals. However, exhibiting intense colouration and conspicuous behaviour might not always be beneficial, for example because such traits also attract competitors and predators. Depending on the context or state of the individual, allocating resources to these traits might thus be costly without leading to a higher mating success. Furthermore, rather than the overall frequency of a behavioural pattern, it might be important for obtaining access to mates to adjust behavioural patterns to the social context, as found in brown-headed cowbirds (*Molothrus ater*) (White et al. 2010). The ability to optimize the expression of social behaviour as a function of available social information, referred to as social competence (Oliveira 2009, Taborsky & Oliveira 2012), can affect the competitiveness and hence the fitness of individuals. More complex social environments may provide increased opportunities for effective social learning and therefore improve individuals' behavioural performances (White et al. 2010, Gersick et al. 2012).

In our study species, the zebra finch (*Taeniopygia guttata*), a multitude of environmental effects during adolescence on adult sexual behaviour and morphological parameters have been revealed (Jones et al. 1996, Leader & Nottebohm 2006, Mariette et al. 2013, Ruploh et al. 2013, Honarmand et al. 2015, Bölting & von Engelhardt 2017 (chapter 2), unpublished (chapter 3)). Zebra finches are highly social, monogamous passerines that form life-long pair bonds and display biparental care. They may reproduce as early as at 60 days of age, although the median age for reproduction is 90 days (Zann 1996). Notably, the average life span of wild zebra finches is relatively short, with an estimated life expectancy of 51 days at hatching (Zann 1996). Therefore, under natural conditions, many individuals only get the chance to reproduce once, if at all. The longevity of birds in captivity is much higher, but both natural and captive populations suffer from high rates of clutch failure (Fenske & Burley 1995, Millam et al. 2001). In order to increase their reproductive success, zebra finches may hence use the alternative reproductive strategies of conspecific brood parasitism (CBP) and extra pair paternity (EPP), even though the relevance under natural conditions has been questioned, since especially EPP are only rarely found in the wild (Birkhead et al. 1990, Griffith et al. 2010, Schielzeth & Bolund 2010). In captivity, it has been shown that more attractive males may accrue increased fitness gains through allocation to EPPs compared to less attractive males (Burley et al. 1996) and it seems similarly likely that more competitive males have a higher chance to engage in extra-pair mating than less competitive males.

Recent experiments in our lab strongly suggest that the attractiveness and competitiveness of zebra finch males are affected by the social environment experienced during adolescence. In the presence of females in adulthood, males reared in groups composed only of juveniles during adolescence show more courtship singing than males reared in mixed-age groups or in juvenile pairs (Bölting & von Engelhardt 2017 (chapter 2), unpublished (chapter 3)). In addition, in sexual contests, males reared in groups, but not males reared in pairs, adjusted

their level of aggression to their opponents' fighting skills (Bölting & von Engelhardt 2017 (chapter 2), unpublished (chapter 3)). Especially when there is high potential for females to be choosy during mate choice and for male-male competition, individuals exhibiting high rates of courtship and increased competitiveness may have a reproductive advantage, as the courtship rate is under directional sexual selection in zebra finches (Collins et al. 1994, Zann 1996) and females prefer to mate with dominant males (Zann 1996). The potential for female choosiness and for male-male competition is likely to be increased in a more complex social environment, as more individuals try to gain access to the best quality partners available. Hence, increased attractiveness and competitiveness of group-reared males might represent adaptive phenotypic modifications to enriched social environmental conditions.

However, evidence for increased fitness gains of group-reared males in enriched social environments is missing. Therefore, the aim of the present study was to investigate the effects of the social environment during adolescence on the reproductive success of males. An experimental design resulting in high competition was chosen, as differences in courtship and aggression are most likely to have the strongest effect under these conditions. We predicted that in a female-restricted breeding experiment, males reared in groups would have increased reproductive success compared to males reared in pairs. However, we predicted that males from juvenile groups and mixed-age groups might also differ in reproductive success due to differences in courtship singing between males potentially affecting their attractiveness. We further predicted that the increased reproductive success of group-reared males might partly be due to increased success in obtaining paternities with multiple females, an indicator for the occurrence of extra-pair mating.

Methods

Subjects and housing conditions

As experimental subjects, male domesticated zebra finches (*Taeniopygia guttata*) bred at the University of Bielefeld were used. These males were born and housed in mixed-sex mixed-age groups (composed of 8-16 adult females, 8-16 adult males and their offspring) in aviaries (350 cm high x 120-240 cm wide x 300 cm deep) with unrestricted visual and auditory contact to other birds until the beginning of the adolescent period. Housing took place under a controlled 14:10 h light: dark cycle (lights on at 7:00 am) with additional daylight entering through glass windows. At the start of adolescence (average age 41 days, range: 36-45), males were assigned to three different social treatments: juvenile pairs (one juvenile male and one juvenile female), juvenile groups (three juvenile males and three juvenile females), and mixed-age groups (three juvenile males, three juvenile females, two adult males and two adult females). All birds assigned to the same treatment group were unfamiliar to each other.

Allocation of siblings over the three social treatments was randomized, so that brothers were allocated to different social treatments and groups. Adults assigned to the mixed-age groups originated from our breeding stock and were mated pairs. During the treatment period, males were kept in small aviaries (200 cm high x 100 cm wide x 100 cm deep) and could hear, but not see birds in adjacent aviaries. Furthermore, birds received no natural daylight, but had fluorescent full-spectrum light tubes (Osram, Biolux, L58W/965). After the end of the adolescent period (average age 110 days, range: 104-114), all males were housed individually in cages (30 cm high x 40 cm wide x 40 cm deep), following previously used protocols (Ruploh et al. 2013, Bischof et al. 2002). Cages were placed in the same room where males had hatched and without visual contact between birds. Start and end of adolescence were chosen according to previous studies (Adkins-Regan & Leung 2006, Ruploh et al. 2013) and were based on typical morphological, behavioural and physiological changes of individuals occurring during that time (Sossinka & Böhner 1980, Zann 1996). Seed food and water was provided *ad libitum* throughout, and this diet was regularly supplemented by germinating seeds, fresh greens and a water bath.

Earlier use of experimental males

Experimental males of the present study had been subjected to several experimental procedures during the time in the social treatments and in early adulthood. The frequencies of males' social interactions and songs were recorded three times during adolescence, using focal animal sampling and continuous recording (Martin & Bateson 1993). Blood samples were taken three times to measure changes in testosterone and corticosterone levels during development. The colouration of the males' beak, cheek patches and breast stripes was visually scored once a week to assess the development of the adult male colouration. To analyse the weight gain of males, their body mass was measured five times during adolescence. In adulthood, the males' courtship and aggressive behaviour was tested in different contexts. Male courtship song towards a single unfamiliar female (average age: 158 days, range: 152-161) was recorded during 10 min by attaching a cage with such an unfamiliar female to the front of a male's cage. By placing two males from different social rearing environments together in a cage with an unfamiliar female for 60 min, male directed aggressive behaviour of focal males was measured as a function of the social background of the opponent (average age: 165 days, range: 161-169). The ability of males to adjust courtship singing to the mating status of females was analysed at an average age of 328 days (range: 283-371), by introducing males singly into an unfamiliar flock of conspecifics comprising two pairs of pair-bonded females and males as well as two unpaired females for 24 hours. In the same set up, aggressive interactions of focal males with the mated males were recorded. For further details on the experimental procedures, see Bølting & von

Engelhardt 2017 (chapter 2), unpublished (chapter 3). After the last experiment, all males were housed together in a big aviary (350 cm high x 240 cm wide x 300 cm deep) with unrestricted visual and auditory contact to other birds until the start of the breeding experiment.

Breeding experiment

In order to examine whether the social environment during adolescence affects the reproductive success of males, 30 males (10 from each social rearing condition) were placed together with 20 females in a big aviary (350 cm high x 360 cm wide x 300 cm deep). When possible, trios and pairs of brothers reared under different social conditions during adolescence were chosen to control for male relatedness. We thus used 3 trios (each brother from a different social condition) and 5 pairs of brothers (1 pair of brothers from a juvenile pair and a juvenile group, and 4 pairs of brothers from a juvenile group and a mixed-age group). The remaining 11 males had no brothers in the experiment (6 males from juvenile pairs, 2 males from juvenile groups, 3 males from mixed-age groups). To reduce competition over nest sites, the aviary was equipped with a total of 45 nest boxes, fixed at a height of 150-300 cm, so that on average 1.5 nest boxes were available per male.

The experiment started at an average age of 655 days (range: 630-678), when males were weighed, and their tarsus length and wing length were measured. They were then ringed with a unique-combination of colour rings and released into the aviary.

A day before males were selected, 20 females from our lab stock housed in a single-sex aviary were caught, a small blood sample (<10 µl) for paternity analysis was taken and females were housed in groups of four in standard double cages (30 cm high x 80 cm wide x 40 cm deep). On the following day, females underwent the same measurements as males did. Females were introduced into the aviary of males a week later to ensure recovery from catching, blood sampling and morphological measurements. All females used were unfamiliar to all experimental males.

On the day on which females were placed into the aviary of males, coconut fibres were distributed on the aviary floor. These serve as nesting material for zebra finches and were replenished daily. Nest boxes were checked daily to record the number of eggs and offspring. Nestlings were ringed at an average age of 12 days (range: 10-14). All birds of the breeding experiment were caught when all offspring were nutritionally independent (age of youngest offspring at catching: 36 days) and blood samples were taken from all offspring and experimental males for paternity analyses. A tissue sample was taken and used for paternity analyses from all offspring that died before a blood sample could be taken (n = 2). Paternity analyses were conducted on a total of 41 chicks that hatched.

Paternity analysis

Tissue samples of dead chicks were stored in 70% ethanol. Blood samples (<10 µl) of the other birds were collected in heparinised capillaries after puncturing the ulnar vein with a hypodermic 26-gauge needle and were stored in phosphate buffered saline (PBS) (10 mM PBS+6 mM EDTA, pH 7.4) at -20 °C until further processing.

Total genomic DNA was extracted using a standard phenol-chloroform extraction method. Samples were then genotyped using 14 polymorphic microsatellite loci previously developed and verified for the zebra finch (Dawson et al. 2005, Forstmeier et al. 2007): Tgu1, Tgu3, Tgu4, Tgu5, Tgu6, Tgu7, Tgu8, Tgu9, Tgu10, Tgu11, Tgu12, Pdou5, Indig41, Ase50. A Type-it Mastermix PCR (polymerase chain reaction) kit (QIAGEN) was used for genotyping. Each 10-µl PCR contained 1 µl (10-60 ng) of DNA, 5 µl of the Type-it Mastermix (containing 6 mM MgCl₂), 3 µl of ddH₂O, and 1 µl of one of the following primer mixes. Primer mix 1 contained 2 µM each of forward and reverse primer of Tgu3, Tgu7, Tgu9, Tgu11 and Pdou5. Primer mix 2 contained 2 µM primer of Tgu4, Tgu6, Tgu8, Tgu10, Tgu12 and Ase50. Primer mix 3 contained 2 µM primer of Tgu1, Tgu5 and Indig41. The following PCR protocol was used for primer mix 1 and primer mix 2: initial denaturation at 95°C for 5 min, 8 cycles denaturation at 95°C for 30 s, 8 cycles annealing at 62°C for 90 s (reduced 1°C per cycle) and extension at 72°C for 60 s, followed by 21 cycles denaturation at 95°C for 30 s, annealing at 58°C for 90 s and extension at 72°C for 60 s. A final extension was performed at 70°C for 15 min. For primer mix 3 the same PCR protocol was used, except that the first annealing step was performed at 60°C and the second annealing step at 56°C.

Fragment sizes were automatically scored using the software GeneMarker V2.6.2 (SoftGenetics LLC®). All scores were verified manually and adjusted in case the genotype call was deemed to be an error. Each male and each female were characterized by a unique genotype and could thus be used for the assignment of paternities. Paternities were assigned by applying the automated Fortran programme COLONY 2.0.6.1 (Zoological Society of London) and the outcome was manually verified.

Out of the 41 chicks that hatched, paternities could not unambiguously be assigned for 3 chicks from 3 different nests, due to more than two mismatches with potential fathers. Therefore the final sample size was 38 or 41, dependent on the variable of interest.

Using the 38 offspring for which paternities could be assigned, the following variables regarding the reproductive performance of males from different social treatments were analysed: *Proportion of males with offspring*: Proportion of males producing offspring from all males of a social treatment. *Number of offspring*: the mean number of offspring sired by a male. *Number of nests with offspring*: the mean number of nests in which offspring sired by a male hatched.

For the analyses of the following variables, all 41 chicks were used, thus including the three chicks with unassigned paternities. *Paternity success*: the proportion of offspring that a male sired in nests in which he sired at least one offspring. *Exclusive paternity*: the proportion of nests with own offspring in which all offspring were exclusively sired by a male. *Shared paternity*: the proportion of nests with own offspring in which offspring were sired by more than one male.

Data analysis

In order to be able to perform parametric statistical analyses, some data had to be transformed to achieve equal variances between social treatment groups and normally distributed residuals. Variances and distributions were assessed visually using variance plots, histograms of residuals and Q-Q plots. For some data, no transformation could be applied that achieved equal variances and normally distributed residuals. These data were therefore analysed non-parametrically using Kruskal-Wallis tests and pairwise Wilcoxon tests, although these could not take into account random effects of male ID and male family ID. Significances of results did not differ between non-parametric and parametric analyses and did not change with transformation method applied. Therefore only the parametric statistics are presented in the figures and text.

Morphology data (weight, tarsus length, wing length) met the criteria of variance homogeneity and normally distributed residuals without transformation. The *number of offspring* of experimental males and the *number of nests with offspring* of experimental males were analysed after a square root transformation. Morphology data, the number of offspring and the number of nests with offspring were analysed by linear mixed models (LMM), assuming a normal distribution.

The *proportion of males with offspring*, *paternity success*, and the proportions of *exclusive paternity* and *shared paternity* of experimental males were analysed by a generalised linear mixed model (GLMM), using a binomial distribution and a logit link function.

Data were analysed in R 3.1.2 by mixed effects models using a maximum likelihood approach (package lme4). Significances of effects were calculated using likelihood ratio tests.

All analyses included a random effect of the male's family ("nest ID") to control for non-independence of measures from brothers allocated to different treatment groups. In addition, analyses for data with multiple measures of each male (*number of offspring*, *proportion own offspring*) included a random effect of the male's identity ("male ID") to control for non-independence of those measures.

All analyses were performed with the social treatment during adolescence ("social treatment") as the only fixed effect. Even when not significant, the main effect of "social

treatment” was always kept in the final model. Whenever there was a significant main effect of “social treatment” in the model, subsequent pairwise comparisons between two experimental groups were conducted. Post-hoc tests were conducted using Sidak adjustments to account for multiple testing. The significance level α was set at $p < 0.05$.

There was only a single male reared in a juvenile pair that reproduced and he sired only a single chick in a single nest. Therefore, differences in the proportion exclusive and shared paternity of nests and offspring were compared only between males reared in juvenile groups and males reared in mixed-age groups. Graphs show the means \pm standard error (SE) estimated with “social treatment” as a fixed effect, and including the random effects.

Results

Male morphology

There was a significant effect of the social treatment during adolescence on the weight of males before the start of the breeding experiment (LMM: social treatment: $\chi^2 = 7.56$, $df = 2$, $p < 0.03$). Males from juvenile pairs weighed significantly less than males from juvenile groups ($\chi^2 = 5.80$, $df = 1$, $p < 0.05$), whereas males from the other treatments did not differ significantly from each other ($\chi^2 < 2.92$, $df = 1$, $p > 0.24$).

Tarsus length (LMM: social treatment: $\chi^2 = 4.18$, $df = 2$, $p > 0.12$) and wing length (LMM: social treatment: $\chi^2 = 2.07$, $df = 2$, $p > 0.35$) of males were not significantly influenced by the social treatment during adolescence.

Proportion of males with offspring

In total, 13 of 30 experimental males (43.3 %) sired offspring in the present study. 1 male (7.7%) was reared in a juvenile pair during adolescence, 6 males (46.2%) were reared in a juvenile group and 6 males (46.2%) were reared in a mixed-age group.

The social treatment during adolescence significantly influenced the proportion of experimental males that sired offspring (GLMM: social treatment: $\chi^2 = 7.63$, $df = 2$, $p < 0.03$). Significantly fewer males reared in juvenile pairs had offspring compared to males reared in juvenile groups or in mixed-age groups (GLMM: social treatment: $\chi^2 = 5.94$, $df = 1$, $p < 0.05$), while group-reared males did not differ from each other (GLMM: social treatment: $\chi^2 = 0.00$, $df = 1$, $p = 1.0$).

Number of offspring

Paternities could be assigned for 38 of 41 offspring. Males reared in a juvenile pair sired 1 of 38 (2.6%) offspring, males reared in a juvenile group sired 18 of 38 (47.4%) offspring, and males reared in a mixed-age group sired 19 of 38 (50.0%) offspring.

The number of offspring sired by experimental males was significantly influenced by their social treatment during adolescence (LMM: social treatment: $\chi^2 = 8.44$, $df = 2$, $p < 0.02$; Fig. 1A). Males reared in juvenile pairs during adolescence sired significantly fewer offspring than males reared in juvenile groups or mixed-age groups (LMM: social treatment: $\chi^2 > 7.04$, $df = 1$, $p < 0.03$), while there was no significant difference between males from the group treatments (LMM: social treatment: $\chi^2 = 0.00$, $df = 1$, $p = 1.0$).

Number of nests with offspring

The 38 offspring came from 15 out of 45 (33.3%) nests. Pair-reared males sired offspring in only 1 of 15 (6.7%) nests, while males from juvenile groups sired offspring in 9 of 15 (60.0%) nests and males from mixed-age groups sired offspring in 10 of 15 (66.7%) nests.

The number of nests with offspring sired by experimental males was significantly influenced by their social treatment during adolescence (LMM: social treatment: $\chi^2 = 7.89$, $df = 2$, $p < 0.02$; Fig. 1B). Males reared in a juvenile pair sired offspring in significantly fewer nests than males reared in a juvenile group or males reared in a mixed-age group (LMM: social treatment: $\chi^2 > 6.59$, $df = 1$, $p < 0.03$). Males from juvenile groups and mixed-age groups did

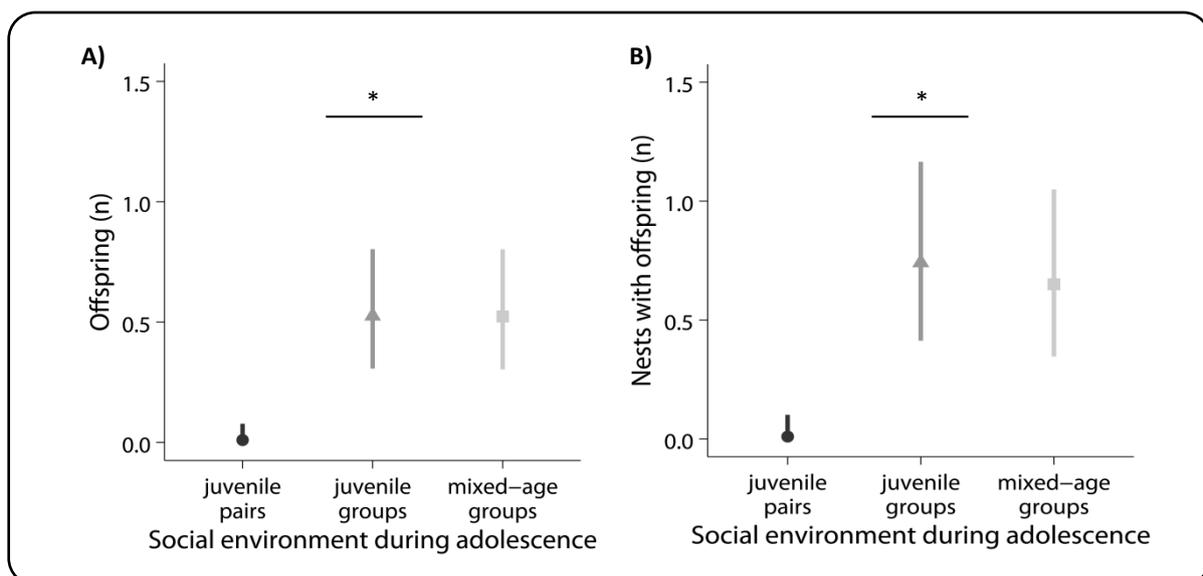


Fig. 1: Reproductive success of experimental males. **A)** The number of offspring and **B)** the number of nests with offspring sired by experimental males was significantly influenced by their social treatment during adolescence. For details, see text. Shown are means \pm SE.

not differ significantly from each other in the number of nests in which they sired offspring (LMM: social treatment: $\chi^2 = 0.03$, $df = 1$, $p > 0.86$).

Paternity success and exclusive vs. shared paternities

In 5 of 15 (33.3%) nests, representing 9 of 38 offspring (23.7%), all offspring in a nest were sired by only one father, while in 10 of 15 (66.7%) nests, representing 29 of 38 chicks (76.3%), there were 2 to 4 different fathers.

Males from juvenile pairs sired in total only 1 chick in 1 nest in which no offspring sired by another male was present and are therefore not considered in the following analyses. Males from juvenile groups sired 18 offspring in 9 nests, of which 1 chick (5.6%) hatched in 1 nest (11.1%) in which no offspring was sired by another male, and 17 chicks (94.4%) hatched in 8 nests (88.9%) that contained a total of 26 offspring from more than one father. Males from mixed-age groups sired 19 offspring in 10 nests, of which 7 chicks (36.8%) hatched in 3 nests (30.0%) in which no offspring was sired by another male, and 12 chicks (63.2%) hatched in 7 nests (70.0%) with a total of 24 offspring from more than one father.

Males reared in juvenile groups and males reared in mixed-age groups did not differ significantly in the proportion of offspring they sired in all nests with own offspring (*Paternity success*: GLMM: social treatment: $\chi^2 = 0.19$, $df = 1$, $p > 0.66$). Furthermore, they did not differ significantly in the proportion of nests in which they exclusively sired all offspring (*Exclusive paternity*: GLMM: social treatment: $\chi^2 = 1.44$, $df = 1$, $p > 0.23$) and thus also not in the proportion of nests in which they lost paternity to at least one other male (*Shared paternity*: GLMM: social treatment: $\chi^2 = 1.44$, $df = 1$, $p > 0.23$).

Discussion

Differences in courtship behaviour and aggressiveness induced by the social environment during adolescence have been suggested to represent behavioural adaptations to future environmental conditions in mammals. Here, we present first-time evidence that such behavioural shaping during adolescence is potentially adaptive in a bird species, the zebra finch (*Taeniopygia guttata*). We demonstrate that males reared in mixed-sex groups have higher reproductive success than males reared in mixed-sex pairs when breeding in an environment with high potential for female choosiness during mate choice and for male-male competition. More males reared in juvenile groups or mixed-age groups had offspring than males reared in juvenile pairs. In addition, group-reared males sired a larger number of offspring and in a larger number of nests than pair-reared males, which had only a single offspring in a single nest. There were no differences between males reared in groups with and without adults in reproductive parameters. These findings strongly suggest that males

reared in the adolescent social environment of a group are, compared to males reared in a pair, better adapted to a life in a complex social environment with increased competition in adulthood.

Reproductive success of males

As proposed by the Predictive Adaptive Response hypothesis, environmental cues during early development may induce phenotypic modifications in individuals that provide a fitness advantage when individuals encounter the predicted environmental conditions later in life (Bateson et al. 2014). Until now, most research regarding adaptive behavioural shaping by the social environment focused on the effects of the prenatal or early postnatal environment (e.g. Kaiser & Sachser 2005, Branchi 2009). Even though it has been proposed that the social environment during adolescence might be a more reliable predictor of future environmental conditions, evidence for adaptive behavioural shaping by the social environment during adolescence is still scarce (Sachser et al. 2011, Zimmermann et al. 2017).

We suggest that in the present study zebra finch males reared in groups during adolescence gained increased reproductive success compared to pair-reared males because they showed higher rates of courtship and aggressive behaviour and were more skilled in adjusting their behaviour to the social context, thereby enhancing their attractiveness and competitiveness (Barlow 1986, Collins et al. 1994, Doutrelant & McGregor 2000, White et al. 2010, Gersick et al. 2012, Taborsky & Oliveira 2012, Clutton-Brock & Huchard 2013). Even though we did not measure courtship and aggressive behaviour again, we expect that males still behaved similarly in the present experiment as in earlier studies with the same birds (Bölting & von Engelhardt 2017 (chapter 2), unpublished (chapter 3)), since the previously detected differences were stable over several months. More attractive, competitive and skilled males may outperform rivals, attract female mates and successfully reproduce especially when there is high mate-competition within a social group. For example, directing aggression towards inferior opponents, as did group-reared males (Bölting & von Engelhardt 2017 (chapter 2), unpublished (chapter 3)), is likely to increase the males' mating opportunities, whereas aggressively attacking males that are superior would be waste of energy or even risk physical injury. Similarly, aggressive behaviour towards male rivals instead of females (Bölting & von Engelhardt unpublished (chapter 3)) is likely to increase the chance to mate. The more complex a social group is, the more likely it is that the potential for female choosiness during mate choice and for male-male competition is increased. Therefore, the enriched social rearing environments of a juvenile group or mixed-age group seem to have prepared experimental males for a life under environmental conditions of high mate-competition in adulthood. A more complex social environment has also been shown to

improve the behavioural performance of individuals in a competitive situation in other species. Cichlid fish fry (*Neolamprologus pulcher*) that had been reared with peers and adults showed more appropriate aggressive responses to experimentally assigned social roles than fry that had been reared with peers only (Arnold & Taborsky 2010). Even though not explicitly tested, it was suggested that the social skills of individuals reared with older and same-age conspecifics may reduce the costs of contests and thus increase fitness. In brown-headed cowbirds (*Molothrus ater*) individuals from dynamic social environments used aggression and courtship towards unfamiliar conspecifics more selectively than males from stable social environments, resulting in increased mating success (White et al. 2010, Gersick et al. 2012).

Attractiveness and competitiveness of the zebra finch males reared in groups during adolescence may also have been increased by morphological traits. Males from juvenile groups weighed more than males from juvenile pairs, although they were not larger since tarsus and wing length did not differ from males from the other rearing environments. In many taxa, individuals with increased body weight often outcompete individuals that weigh less in physical contests (Andersson 1994, Kotiaho et al. 1997). However, males from mixed-age groups also had increased reproductive success compared to pair-reared males, but did not weigh more than those. Yet, males from mixed-age groups developed the adult male colouration significantly faster during adolescence than males from the other treatments (Bölting & von Engelhardt 2017 (chapter2)). Even though no differences in degree of colouration were found during early adulthood, males might differ in colour intensity. Colour intensity was not measured here, but has been found to be affected by the social environment, though during adulthood, in another passerine, the blue-black grassquit (*Volatinia jacarina*), with consequences for male attractiveness (Maia et al. 2012).

While several studies report that a more complex social environment during development improves the behavioural performance of individuals, such improved performance can be due to both an increase and a reduction in courtship and aggressive behaviour. Increased fitness may thus result from either change in behaviour, depending on the social or ecological context in which individuals reproduce or the social or mating system of the species. In polygamous social species, for example, in which one male monopolizes several females, as in guinea pigs (*Cavia aperea f. porcellus*), young have been suggested to benefit from a “camouflage” or “queuing” strategy, i.e. a low-aggression phenotype, when social density is high (Kaiser & Sachser 2001, Sachser et al. 2011, 2013). In contrast, when social density is low, young guinea pigs might rather benefit from a “fighting / resource defence” strategy, i.e. a high-aggression phenotype (Sachser et al. 2013, Zimmermann et al. 2017).

To our knowledge our study provides the first evidence for such adaptive behavioural shaping in zebra finches. Surprisingly, earlier studies found a different pattern for effects of

the social environment during adolescence on the courtship and aggressive behaviour of zebra finch males (Ruploh et al. 2013, Ruploh et al. 2014b). These studies suggested that reduced courtship and aggressive behaviour at higher social densities would have similar adaptive benefits in zebra finches as in guinea pigs. A reduction in courtship and competition under increased social density would lead to reduced social conflicts (Ruploh et al. 2013, Sachser et al. 2011). Differences between the current and earlier studies are not yet explicable, but might have been caused by the acoustic environment or photoperiod (for a detailed discussion, see Bølting & von Engelhardt 2017 (chapter 2)). Clearly, further studies are needed to understand whether the long-lasting effects of the social environment during adolescence on zebra finches are indeed adaptations to variation in the social environment during adulthood or whether enriched social conditions during early life are always favourable and provide an advantage (“silver-spoon effect”) (Grafen 1988). It will be especially important to demonstrate that group-reared zebra finch males are outperformed by pair-reared males in a social environment of low complexity and with low potential for female mate choice and male-male competition, as well as to figure out whether similar effects as those found here are observed under natural conditions.

Incidence of multiple paternities

Several males obtained paternities in several nests, strongly indicating the occurrence of extra-pair paternities (EPPs), although pair bonds were not assessed in the present study. However, males reared in groups of only juveniles and males reared in mixed-aged groups did not differ in paternity success in nests or in the proportion of nests with exclusive and shared paternity. This suggests that there was no difference in reproductive strategies between group-reared males. A comparison with pair-reared males was unfortunately not possible since only a single pair-reared male reproduced, producing a single offspring.

In many monogamous species, males may increase reproductive success through extra-pair copulations (Griffith et al. 2002), which may be an alternative male reproductive strategy depending on social density and male quality. When social density is high, it seems likely that there are increased opportunities to engage in extra-pair mating. Furthermore, in zebra finches (Houtman 1992, Burley et al. 1994, 1996) and other species (Kempnaers et al. 1997) increased attractiveness facilitates EPPs and increased competitiveness is also likely to enhance the chance to mate with several females. Increased attractiveness and competitiveness might also reduce the risk of males to lose paternity to rivals. Females mated to attractive males are less likely to engage in extra-pair mating than females mated to unattractive males (Burley et al. 1994) and a high competitiveness may enable males to more successfully prohibit copulatory attempts of extra-pair males with their own female. The results of the present study may indicate that both group-rearing environments similarly

influenced both the attractiveness and competitiveness of males. This seems a bit surprising, however, as only males reared in juvenile groups had previously been found to show an increased level of courtship singing towards females (Bölting & von Engelhardt 2017 (chapter 2)), a trait that is under direct sexual selection in zebra finches (Collins et al. 1994, Zann 1996). Therefore, it is possible that the presence of or contact with adults in the rearing environment significantly affects the behavioural performance of individuals in zebra finches (Adkins-Regan & Krakauer 2010, Bölting & von Engelhardt 2017 (chapter 2)) and other species (West & King 1988, Delville et al. 2003, Arnold & Taborsky 2010), but that this only results in fitness differences under specific environmental conditions in adulthood. In the experimental set up chosen in this study, increased competitiveness might have been the most important trait of males facilitating access to females. Further studies should investigate this in more detail.

In general, EPPs can be especially beneficial in species displaying biparental care, as individuals increase their reproductive success without having the costs of parental investment (Burley et al. 1996). In the zebra finch, both males and females care for their young (Zann 1996). However, even though EPPs are observed frequently (28%) in captive studies of zebra finches (Burley et al. 1996), they occur only very rarely (1.7% - 2.4%) under natural conditions (Birkhead et al. 1990, Griffith et al. 2010). Therefore, zebra finches have been suggested to more closely approach true monogamy than any other bird species (Griffith et al. 2010) and the reason for the occurrence of EPPs in captivity is not yet clear. It might be that in captivity, females are just more likely to accept extra-pair courtship from attractive males (Griffith et al. 2010), because artificially assigned mating partners do often not match the selective preferences of birds and extra-pair mating might compensate, for example, genetic disadvantages or infertility of a partner to at least some degree (Sheldon 1994). The importance of the pair bond for the occurrence of EPPs has also been referred to in a study about a close relative of the zebra finch, the monogamous long-tailed finch (*Poephila acuticauda*) (van Rooij et al. 2016). In addition, in zebra finches, pair-bonding experience significantly impacts on males' fitness (Mariette et al. 2013).

Overall, both group-rearing environments during adolescence may affect the reproductive success of males, including EPPs, only under the breeding conditions used in the present study. More studies are therefore needed to investigate the consequences of the age-structure of the social rearing environment during adolescence on reproductive success of males in different adult environments. In addition, future studies should investigate whether pair-bonding status or pair-bond quality significantly affects reproductive strategies of individuals.

Conclusion

Until now, most research concerning adaptive behavioural shaping focused on the effects of environmental cues during the prenatal or early postnatal environment. Our study shows that rearing zebra finch males in more complex social environments during adolescence, which leads to increased courtship singing and increased fighting skills, also enhances mating success under complex environmental conditions in adulthood. This supports the idea that social cues during adolescence can induce adaptive behavioural modifications with consequences for fitness, which has so far been only reported for mammals

Chapter 5

General Discussion

S. Bölting

In addition to the prenatal and early postnatal life stages, adolescence has recently been considered to be another developmental period in which phenotypic traits can be significantly affected by the social environment. However, until now, the underlying mechanisms and fitness consequences of phenotypic variation induced by the social environment during adolescence have only been investigated in some mammals and hardly at all in birds. I investigated in this thesis in a bird species, the zebra finch, whether effects of the social environment during adolescence on adult social behaviour might be mediated via differences in social interactions affecting endocrinological, morphological, and behavioural parameters during maturation. In addition, I tested whether adult behavioural differences represent adaptive modifications. Male zebra finches were reared in mixed-sex juvenile pairs (one juvenile male and one juvenile female), mixed-sex juvenile groups (three juvenile males and three juvenile females) or mixed-sex mixed-age groups (three juvenile males, three juvenile females, two adult males and two adult females) during adolescence in order to compare the effects of different levels of social complexity on developmental and adult traits. My experiments revealed a significant and stable effect of the adolescent social environment on adult courtship and aggressive behaviour of males (chapter 2 & 3) and hence confirmed the importance of adolescence as a sensitive window for phenotypic shaping. Extending previous findings, I found that the effects of the social environment during adolescence on the behavioural performance of zebra finch males in adulthood depend on the characteristics of interaction partners, such as their sex or their own social rearing conditions (chapter 2 & 3). My results suggest that adult behavioural modifications depend on changes in social interactions affecting behavioural and morphological maturation. However, there was no evidence for long-lasting effects of endocrinological changes during maturation (chapter 2). Last, I found that males reared in more complex social environments during adolescence gained increased reproductive success in a complex social environment in adulthood (chapter 4). Taken together, these results indicate that the social environment during adolescence influences phenotypic variation of zebra finch males via effects on both developmental plasticity and phenotypic flexibility and that the induced behavioural modifications are adaptive.

In the following, I discuss my findings in a broader context and present ideas for future research that might contribute to a better understanding of the relationship between the social environment, social interactions, endocrine changes and adult behaviour. Furthermore, I make suggestions how the adaptive value of behavioural modifications can be demonstrated.

Phenotypic plasticity: effects of the social environment during adolescence

As illustrated by the experiments conducted in this thesis, the social environment during adolescence exerts significant effects on morphological and behavioural traits of zebra finch males. Some of the induced modifications are likely to represent examples for developmental plasticity, while others demonstrate effects on phenotypic flexibility (Piersma & Drent 2003).

The accelerated development of the adult plumage colouration in males from mixed-age groups compared to males from the other treatments, presented in chapter 2, indicates a faster maturation of males from mixed-age groups. Similarly, increased levels of plastic song (Immelmann 1969, Scharff & Nottebohm 1991, Tchernichovski et al. 2001) produced by males reared in juvenile groups and males reared in mixed-age groups compared to males reared in juvenile pairs during early adolescence indicate that group-reared males mature faster. Both the moult into adult plumage as well as the development of adult song are developmental transitions experienced by young zebra finches during sexual maturation (Zann 1996). Differences in the age at sexual maturity between individuals are a typical example of developmental plasticity that can be highly relevant for fitness. In other species, for example wild covies (*Cavia aperea*), the age at sexual maturity has also been shown to be affected by environmental factors during development (Trillmich et al. 2009, Guenther et al. 2014). In wild zebra finches, the average life expectancy of individuals is 51 days, but reproduction occurs not before 60 days of age, with 90 days being the median age of first reproduction (Zann 1996). Therefore, under natural conditions, many individuals may only reproduce once, or not at all. The earlier individuals attain sexual maturity, the higher their lifetime reproductive success may be. Future studies may confirm the effect of the social environment during adolescence on the age at sexual maturity in zebra finches by, for example, more frequently observing them during adolescence for the occurrence of clear signs of the onset of sexual maturity, such as the first mounting and copulation attempt or the first successful fertilisation of an egg laid by a female mate after copulation.

While the morphological modifications induced by the social environment during adolescence are examples for developmental plasticity, the variation in behavioural traits demonstrates differences in phenotypic flexibility between males. In chapter 2, I report that males reared in juvenile groups show significantly more courtship singing towards a single female in adulthood than males reared in juvenile pairs. The same effect was observed in a different context in the experiment presented in chapter 3, when males were introduced into an unfamiliar flock of conspecifics several months later. Despite this stable behavioural difference between males from different social treatments, there was variation in the rate of courtship singing, as it decreased significantly over time after introduction into the flock for all

males. Similarly, courtship singing has been found to decrease over time in a previous study on zebra finches from different social rearing environments during adolescence (Ruploh et al. 2014a). Therefore, courtship singing can be considered to represent phenotypic flexibility in zebra finch males (Piersma & Drent 2003). The same is true for aggressive behaviour, as males adjusted their level of aggression towards conspecifics over time and depending on the type of the interaction partner, even though effects differed between social treatments. In the experiment conducted in chapter 2, males reared in groups flexibly adjusted their level of aggression to the social background and hence potentially to the fighting skills of their opponent. Similarly, the experiment conducted in chapter 3 revealed that group-reared males clearly discriminate between male and female interaction partners for aggressive interactions. Even though such an adjustment to the interaction partners' characteristics was not detected in pair-reared males, all males significantly reduced the level of aggressive behaviour towards conspecifics during 24 hours after being introduced into a new flock. Adjustment of aggression over time has also previously been observed for zebra finch males from different social rearing environments during adolescence (Ruploh et al. 2014a). However, evidence that aggression is adjusted in relation to characteristics of interaction partners was missing so far. The increased context-dependent behavioural flexibility shown by males reared in more complex social environments, i.e. groups, is in line with previous findings in other species. In a cichlid fish (*Neolamprologus pulcher*), individuals growing up in an environment with peers and adults are more flexible in adjusting aggressive and submissive behaviour to the social context than fish that were reared with peers only (Arnold & Taborsky 2010). Similarly, in a laboratory strain of house mice (*Mus musculus*), individuals reared in communal nests display a more appropriate use of aggression when in the dominant position and of submission when in the subordinate position during social hierarchy contests later in life compared to individuals reared by only a single mother (Branchi et al. 2006). To my knowledge, the experiments conducted in this thesis are the first to provide evidence for a significant effect of the social environment during adolescence on the ability of individuals to adjust their behaviour to the state of conspecifics in zebra finches. As this ability is a crucial requirement for group living and may significantly influence an individual's fitness (Taborsky & Oliveira 2012), my findings clearly illustrate the importance of social experiences during the life stage of adolescence in social species.

Underlying mechanisms of phenotypic variation: the role of social interactions and hormonal changes

The experiments presented in this thesis revealed a clear effect of the social environment during adolescence on the frequency of social interactions of young. In chapter 2, I have shown that males reared in juvenile groups and mixed-age groups had significantly more social interactions than males reared in juvenile pairs. This is in line with findings in other species, in which individuals exposed to more complex social environments are also involved in more social interactions (Branchi et al. 2006, Arnold & Taborsky 2010, Sachser et al. 2011). Such an increased frequency of social interactions has been considered to be important for behavioural shaping in two ways.

On the one hand, increased social interactions during adolescence seem to significantly influence the development of adult behaviour via the effects on hormones. More precisely, in guinea pig (*Cavia aperea f. porcellus*) males, increased social interactions during adolescence trigger the secretion of testosterone and cortisol, which has been suggested to reduce the adult cortisol responsiveness controlling the display of adult aggressive behaviour via organisational effects (Sachser et al. 2011, 2013). Even though males reared in juvenile groups also exhibited an increase in testosterone levels during late adolescence in my experiment, I cannot confirm the suggested relationship of social interactions, hormonal changes and adult behavioural modifications. First, increased testosterone levels were only found in males from juvenile groups, but not in males from mixed-age groups. This is striking as males from both group-housing conditions experienced an increased frequency of social interactions during adolescence and showed behavioural modifications in adulthood compared to males from juvenile pairs. Second, I found no differences in corticosterone values, the avian equivalent to cortisol, between males from different social treatments. The absence of a peak in testosterone levels during late adolescence in males from mixed-age groups might be explainable by an inhibiting effect of the adult males in that environment (Vandenbergh 1971, Bushman & Burns 1994). Furthermore, social isolation, rather than social stimulation, might increase corticosterone secretion with effects on, for example, vocal activity in the highly social zebra finch (Perez et al. 2012). Even though zebra finch males from different social environments during adolescence did not differ in testosterone and corticosterone levels in my experiments, other hormones might have been affected by increased social interactions in males from both juvenile groups and mixed-age groups, modifying their behavioural and morphological maturation. This is indicated by the increased levels of plastic song shown by group-reared males during early adolescence and the accelerated development of the adult plumage of males from mixed-age groups. Estradiol and luteinizing hormone (LH) are hormones that have been shown to be involved in social

interactions, to affect the development of the neural song system and to regulate plumage colouration (Ralph 1969, Harding 1981, Arnold & Schlinger 1993, Wingfield 1994, Schlinger 1997, Ramage-Healey et al. 2008). Therefore, these are candidate hormones that should be looked at in future studies. Moreover, in order to determine the causal relationship of social interactions during adolescence and hormone secretion in zebra finch males, the frequency of social interactions individuals are exposed to should be strictly controlled in future experiments. This could be done by giving individuals access to interactions partners for only a limited amount of time during development, an approach used previously for example in guinea pigs (Lürzel et al. 2010).

On the other hand, increased social interactions have been considered to significantly affect the development of adult behaviour by providing increased learning opportunities (Bandura 1977, Galef & Laland 2005, Taborsky 2016). First, this means that by interacting more frequently with conspecifics individuals may be better able to fine-tune their behaviours until they are most appropriate for a given situation (Taborsky 2016). With respect to the social treatments applied in my experiments, males reared in both juvenile and mixed-age groups had increased opportunities for behavioural fine-tuning. This might at least partly explain their adult behavioural modifications compared to males reared in juvenile pairs. However, adult behavioural modifications were not exactly the same for males reared in juvenile groups and males reared in mixed-age groups, as only males from juvenile groups sang significantly more to females than males from juvenile pairs. Therefore, I suggest that differences in learning opportunities due to variation in the quality and diversity of social interactions experienced by group-reared males have exerted additional influence. Such differences are likely to be caused by the presence of adults in mixed-age groups. It has also been shown in other species that the age of interaction partners during development crucially influences behaviour of young, with the presence of adults affecting especially the development of sexual behavioural traits, such as song potency in brown headed cowbirds (West & King 1988, White et al. 2002), and thus likely courtship singing rate in zebra finches. A controlled manipulation of the type of interactions individuals experience during adolescence with interaction partners differing in important characteristics such as age, but also e.g. dominance state, would help to more accurately determine their effects on the development of phenotypic variation in zebra finches.

Underlying mechanisms of phenotypic variation: the role of additional factors

Surprisingly, the findings of the experiments conducted in this thesis partially contrast with findings reported in an earlier study on zebra finches that applied two of the same three social treatments during adolescence I used (Ruploh et al. 2013). In the earlier study, zebra finch males reared in juvenile pairs during adolescence showed increased levels of courtship singing towards females and of aggressiveness towards other males in adulthood compared to males reared in juvenile groups. These behavioural modifications closely resembled findings in guinea pigs reared in pairs or groups during adolescence (Sachser et al. 2011, 2013). Therefore, the authors suggested the same underlying mechanisms of the behavioural changes, i.e. a higher frequency of social interactions increasing levels of testosterone and thus reducing adult behavioural displays in group-reared males. Even though neither the frequency of social interactions nor changes in endocrinological parameters in the different environmental conditions were investigated previously, it is very likely that group-reared males experienced increased social stimulation. This suggests that the adolescent shaping process potentially follows a more complex pattern. Most likely, the frequency of social interactions individuals experience differentially affects their development depending on other environmental factors. Environmental factors that crucially affect morphological, physiological and behavioural development, as well as adult behaviour in zebra finches (Slater et al. 1988, Zann 1996, Bentley et al. 2000, Perfito et al. 2007) and other species (Pyter & Nelson 2006, Guenther et al. 2014) are for example acoustic stimulation and the photoperiod. Photoperiodic and acoustic conditions differed between the present and the previous zebra finch studies, therefore potentially explaining the discrepancy in results. Previously, males were housed in outdoor aviaries which were spatially farther apart than the indoor aviaries used in my experiments during adolescence. Most strikingly, effects of photoperiodic conditions may be masked by increased social stimulation or food abundance (Perfito 2010, Perfito et al. 2008). This might explain why pair-reared males in my experiments differed a lot in singing rate or rate of aggression from pair-reared males in previous experiments, while group-reared males exhibited almost similar rates in the different studies (Ruploh et al. 2013, Bötting & von Engelhardt 2017 (chapter 2)). Future experiments should clearly take into account the potential interacting effect of the social environment and photoperiod and / or acoustic stimulation. More standardized experiments eliminating even minor changes in these parameters are needed in order to determine the precise effect of the social environment on adult behaviour.

Sensitive windows for adaptive phenotypic shaping: behavioural modifications influence male reproductive success

A key question regarding variation in phenotypic traits is whether this variation provides fitness benefits to individuals at some point during their life or whether it is a negative consequence of unfavourable influences. The Predictive Adaptive Response hypothesis suggests that environmental cues induce the development of phenotypic traits that are adapted to the demands of an individual's current or future environment (Bateson et al. 2014). While a lot of studies investigated the adaptive significance of phenotypic variation induced during the prenatal or early postnatal period, only recently the life stage of adolescence has started to be in the focus of research. It has been argued that the better individuals become informed about their surrounding environment, the less they should be influenced by new environmental cues (Fawcett & Frankenhuis 2015), making the very early life stages most interesting for research. However, in social species, individuals often experience considerable change in environmental cues beyond the very early life stages, especially during adolescence. Young gain independence from their parents, emigrate from their natal group and increasingly interact with other adults and peers (Baker 1978, Zann 1996, Spear 2000). At the same time, in many species individuals attain sexual maturity (Spear 2000, Sisk & Foster 2004, Sisk & Zehr 2005, Sachser et al. 2011). Therefore, environmental cues during adolescence might more reliably indicate the conditions in which individuals will reproduce or spend the rest of their lives than environmental cues directly after birth or hatching. This may especially apply to monogamous species in which first reproduction may take place shortly after the attainment of sexual maturity and does not have to be delayed until individuals are physically strong enough to compete for monopolisation of mates. In order to comply with changes in the social environment during adolescence and to successfully find a mating partner, individuals have to phenotypically adjust to the new environmental demands. Hence, they still need to be able to acquire knowledge about social rules and to learn how to appropriately behave towards different interaction partners. In doing so, it seems likely that in a more complex social environment, individuals developing the most attractive and competitive traits might have a reproductive advantage, as there might be higher competition for the best mating partner than in a less complex social environment. In zebra finches, intense courtship singing of males is attractive to females and under direct sexual selection (Collins et al. 1994, Zann 1996). It may be obvious that the ability to appropriately use aggression towards opponents to defend a mating partner or breeding territory also facilitates access to mates, as females prefer to mate with dominant males (Zann 1996). Increased courtship singing and ability to adjust aggression was detected in group-reared males in my experiments (chapter 2 & 3). With the

experiment conducted in chapter 4, I found indeed evidence that this variation in courtship and aggressive behaviour induced by the social environment of a group represents adaptive phenotypic modifications to similar environmental conditions in adulthood. When males from all social treatments were simultaneously exposed to an environment with only a limited number of potential female partners, and hence with increased potential for female choosiness during mate choice and for male-male competition, group-reared males attained increased reproductive success compared to pair-reared males. Previously, another potentially fitness relevant consequence of group-rearing during adolescence in zebra finches was detected. Males reared in juvenile groups integrated better into a flock of unfamiliar conspecifics in adulthood than males reared in juvenile pairs (Ruploh et al. 2014a). However, so far it remains unclear whether pair-reared males have fitness benefits compared to group-reared males in a simple social setting in adulthood. This would confirm the adaptive value of phenotypic modifications induced by the social environment during adolescence in zebra finches. In a simple social setting with low potential for mate-competition, early maturation and increased courtship singing to females might unnecessarily waste energy that could have been invested in other important vital functions. In mammals, such as guinea pigs, the adaptiveness of behavioural modifications induced by pair-rearing vs. group-rearing during adolescence has already been confirmed (Sachser et al. 2011, 2013, Zimmermann et al. 2017). Further evidence for adaptive shaping during adolescence in birds would demonstrate that the importance of this life stage as a sensitive window spans across other vertebrate taxa.

Conclusion

The experiments presented in this thesis investigated for the first time in a social bird species, the zebra finch, the mechanisms underlying behavioural variation induced by the social environment during adolescence and the functional consequences of such variation (Fig. 1). It was detected that the frequency of social interactions significantly affects the behavioural and morphological maturation of males, with consequences for adult behavioural performance. Differences in the quality or diversity of interactions with conspecifics of varying age are likely to have exerted additional effects. While previous findings in mammals suggested that effects of social interactions are mainly mediated via changes in testosterone affecting the adult HPA axis responsiveness, the findings presented in this thesis strongly point to mediation via social learning. Nonetheless, other hormones than those investigated here and previously may have been affected by social interactions with consequences for adult behaviour. The findings presented in this thesis further illustrate the complexity of the process of phenotypic shaping and clearly point out the importance to investigate effects of

the social environment during adolescence in relation to other environmental factors. Finally, the effects of adult phenotypic modifications induced by the social environment during adolescence on male reproductive success indicate that they represent adaptations to social environments of similar complexity in later life. This highlights the importance of the social environment during adolescence for the fitness of individuals in social species.

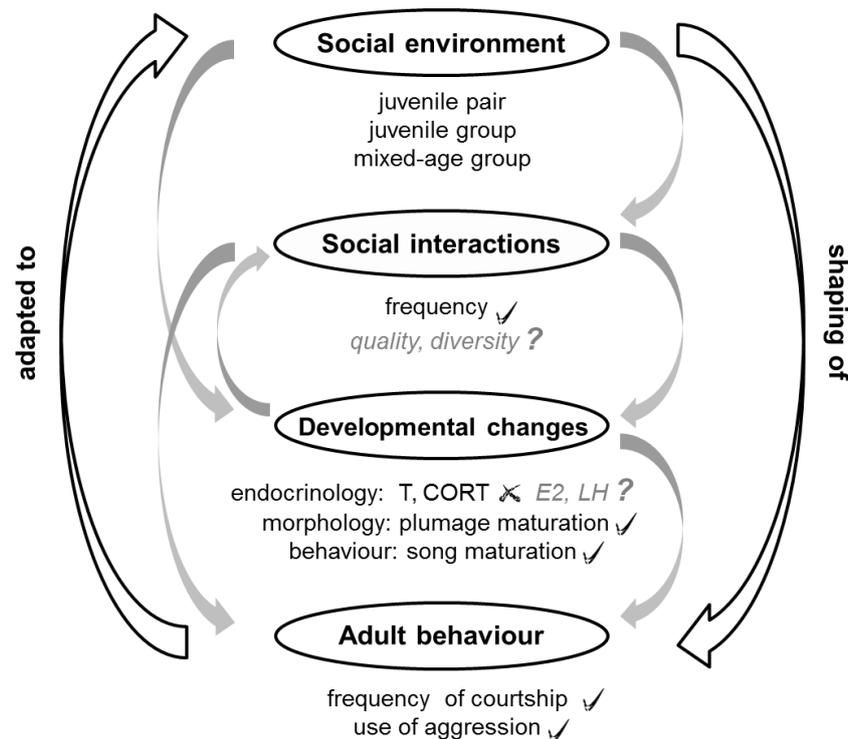


Fig. 1: Graphical summary of the potential mechanisms and consequences of social influences during adolescence on adult behaviour in zebra finches (*Taeniopygia guttata*). The social environment shapes male adult courtship and aggressive behaviour in a way that seems to be adapted to social environments of similar complexity in later life (white arrows). Behavioural modifications are likely induced by differences in the frequency of social interactions, affecting maturation (✓). Long-lasting effects of differences in testosterone (T) or corticosterone (CORT) could not be revealed (✗) (chapter 2 - 4). Possible effects of the quality / diversity of social interactions and changes in estradiol (E2) / luteinizing hormone (LH) levels during adolescence on adult behaviour (?), as well as the causality between the social environment, social interactions, developmental changes and adult behaviour (grey arrows) remain to be investigated.

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Declaration of Originality

By presenting this thesis in fulfilment of the requirements for the academic degree Doctor rerum naturalium (Dr. rer. nat.) at Bielefeld University, I declare that this work is original and has not been submitted for a higher degree or to any other university or institution. I affirm that I have written this thesis by myself and that I have marked all citations and references.

Stefanie Bölting

Bielefeld, October 2017

Erklärung der Urheberschaft

Hiermit versichere ich, dass die vorliegende Arbeit zur Erlangung des akademischen Grades Doktor rerum naturalium (Dr. rer. nat.) der Universität Bielefeld ein Originalwerk ist und nicht an einer anderen Universität oder einem anderen Institut zur Erlangung eines höheren Abschlusses eingereicht wurde. Ich versichere, dass ich die vorliegende Dissertation selbstständig verfasst habe und, dass ich alle benutzten Hilfsmittel und Quellen kenntlich gemacht habe.

Stefanie Bölting

Bielefeld, Oktober 2017

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*It always seems
impossible
until it is done.*

- Nelson Mandela -

