Do mating traits evolve ex situ and should we care?


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Abstract

As a group, birds are facing continued declines in conservation status. As such, ex situ management is becoming a more widely used and important tool in conservation programmes. However, adaptation to captivity and behavioural change during ex situ breeding programmes may reduce the success of these interventions. Changes in mating traits, such as vocal behaviour, may be particularly important. In this thesis, I examine the potential for vocal evolution during ex situ conservation to determine how it could impact conservation efforts, focusing on the Java sparrow (*Lonchura oryzivora*), an endangered estrildid finch. Firstly, I examine the existing literature to determine how vocal behaviour could impact on conservation actions and how potential problems could be mitigated (Chapter 2). Vocal learning and the formation of population dialects have particular significance in conservation programmes due to their effects on important behaviours, such as mate choice. Java sparrows learn a number of components of their song, including song complexity and note acoustic structure (Chapter 3). Birds also learned many temporal features of song, such as tempo, the inheritance of which has been less frequently studied (Chapter 4). Given the importance of vocal learning in song development, there is a high potential for vocal change and population divergence during ex situ breeding in this species. Such differences are important for mate preference; Java sparrow females preferred familiar, over unfamiliar, songs (Chapter 5). Preference for familiar songs may result in assortative mating between individuals of different population origin during conservation efforts, reducing the effectiveness of conservation programmes. As well as behavioural changes, other phenotypic changes may occur in captivity. Captive Java sparrows were both larger and had greater sexual size dimorphism than those of wild origin (Chapter 6). Morphological changes during captive breeding may reduce the performance of captive-bred individuals in wild environments, as well as affecting other, correlated traits, such as vocal behaviour. Vocal behaviour may also be affected by altered sound environments experienced by birds during ex situ breeding programmes. Human presence has a significant effect on the zoo soundscape; reduction in human presence during the COVID-19 lockdown was associated with a number of changes in the sound environment (Chapter 7). Overall, there is significant potential for vocal evolution during the captive breeding of Java sparrows, mediated by cultural processes resulting from social learning of vocalizations, environmental differences, and other, correlated changes. Changes in vocal behaviour in the Java sparrow could affect conservation programmes due to preferences for familiar vocalizations in this species by contributing to assortative mating. The findings from this species suggest that vocal behaviour can have an important role in the success of conservation efforts and requires further investigation in this and other species.

Declaration

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Chapter 1: General Introduction

1.1 The state of birds

Like many taxonomic groups, birds are facing continued declines in conservation status. The Red List Index, based on the number of species that have moved category in the IUCN Red List, shows that the conservation status of birds has deteriorated over the past 30 years (BirdLife International 2022). In 2022, BirdLife International reported that 49% of bird species had declining populations (5,412), whereas only 38% had stable populations (4,234) (BirdLife International 2022). In total, around 1 in 8 bird species (1,409 species) are globally threatened (listed as Vulnerable, Endangered, or Critically Endangered by the IUCN) (BirdLife International 2022). Many species have already faced extinction, with at least 187 species becoming extinct since the year 1500 (BirdLife International 2022).

Birds face a broad range of threats, the most prevalent among which include agricultural expansion, logging and deforestation, invasive species, overexploitation, such as hunting and trapping, and climate change (Bolam et al. 2021; BirdLife International 2022). The effect of these threats differs between groups and is affected by geographic location and life histories. For example, island endemic bird species are more negatively affected by invasive species than mainland birds (Clavero et al. 2009)

1.2 Bird conservation

Birds are a popular and well-studied taxonomic group (Brooks et al. 2008; BirdLife International 2018a, 2020). In line with this, conservation ornithology plays a leading role in the development of conservation science in general (Brooks et al. 2008). Successful conservation efforts require considerable information on target species, and several early attempts to restore populations failed due to a lack of information (Jones 2004). As such, additional research is often needed to fill knowledge gaps for threatened bird species (Jones 2004; Brooks et al. 2008). The importance of evidence-based conservation, where conservation practices are based in scientific evidence, is also becoming more widely recognised and incorporated into conservation programmes (Sutherland et al. 2004; Walsh et al. 2014).

In spite of a clear focus on conservation in recent years, conservation efforts may still not be achieving their targets; a recent assessment by BirdLife International indicated that, when examining data from birds, none of the 2020 Aichi Biodiversity Targets were met (18/20 were examined) (BirdLife International 2020). Despite this, there have been several recent conservation success stories from birds. Bolam et al., (2021) found that conservation efforts had prevented the extinction of 21-32 bird species between 1993 and 2020, suggesting that in the absence of conservation efforts the total number of bird extinctions would have been 3.1-4.2 times higher.

A range of strategies have been employed to further bird conservation. The Important Bird and Biodiversity Area (IBA) Programme has identified over 12,000 sites of international significance, and a number of these sites have been designated protected areas (BirdLife International 2014, 2022). However, currently 36% of these sites are entirely unprotected (BirdLife International 2020). Other broad management strategies may include management of threats, such as disease and predator control (Jones 2004; Smith et al. 2010; Lapointe et al. 2012) or resource provision, such as nesting site provision and supplementary feeding (Elliott et al. 2001; Jones 2004; Libois et al. 2012).

In some cases, broad management strategies, such as protected areas, are not sufficient to prevent extinctions. Intensive management, such as ex situ conservation, reintroductions, and translocations, is a useful tool that has proved valuable in bird conservation (Elliot et al. 2001; Jones 2004; Miskelly & Powlesland 2013; Bolam et al. 2021). Unfortunately, some managed species are now likely to be conservation reliant and unlikely to persist without some form of human input for population or threat management (Scott et al. 1998; Goble et al. 2012).

1.3 Ex situ conservation

Ex situ conservation has the potential to address the causes of primary threats, offset the effects of threats, buy time, and restore wild populations (IUCN Species Survival Commission 2014). Zoos and aquaria are vital hubs for ex situ conservation. However, the populations in these institutions have generally been managed separately from those in situ (Byers et al., 2013). Whereas in situ programmes have aimed to monitor wild populations and develop conservation strategies to conserve species in the wild, ex situ programmes have traditionally aimed to develop long term goals for captive populations (Byers et al., 2013). The Ark paradigm was first described by Soulé et al., (1986) and proposes a role for captive populations in sustaining populations as insurance populations and genetic reservoirs for endangered species. However, ex situ populations have a larger role to play in species conservation. In a recent survey, EAZA (European Association of Zoos and Aquaria) member institutions were found to have made 637 contributions to reintroduction projects (Gilbert et al. 2017). Supplying animals for release was the biggest contribution, with funding, staff, expertise, equipment and coordination also listed. In fact, many of the tools used in ex situ population management in zoos are relevant to in situ conservation, and communication between the two fields could maximize conservation benefits (Byers et al., 2013; IUCN Species Survival Commission, 2014). The One Plan Approach highlights the importance of population management across a continuum of wild and intensively managed conditions, with integrated conservation planning across all responsible parties (Byers et al., 2013). Furthermore, the IUCN Species Survival Commission (2014) suggest that before an ex situ conservation programme is developed or continued, the roles it can play in supporting in situ conservation and the factors that could impede or contribute to conservation success should be considered. Therefore, to ensure the continued success of management strategies, it is important to design effective, well-informed programmes which consider both in and ex situ populations.

1.5 Captive population management

In order to support a more integrated conservation framework incorporating both in and ex situ populations, it is important to ensure that captive populations are appropriately managed so that they are suitable for inclusion in wider conservation programmes. Maintenance of populations in ex situ environments may affect their suitability for in situ conservation programmes and potentially limit the success of interventions. For example, translocations involving captive-bred animals have generally been less successful than those using wild-born animals. Fischer & Lindenmayer (2000) report that whilst 31% of translocations using wild animals were successful, only 13% of reintroductions of captive animals were. This is corroborated by additional studies examining the success of interventions with wild animals vs. animals that spent any period of time in captivity (Griffith et al., 1989: 75% vs 38%; Wolf et al., 1996: 71% vs 50%).

1.5.1 Adaptation to captivity

Captive environments are necessarily different from wild environments. As such, captive populations experience different conditions and thus selection pressures compared to wild populations. These differences can result in adaptation to captivity, a suite of interlinked phenotypic and genetic changes from responses to differing selective pressures, which have been reported in a range of taxa (e.g., Atlantic salmon (Salmo salar) (Bernatchez et al. 2008) Houbara bustard (*Chlamydotis undulata*) (Chargé et al. 2014), orange-bellied parrot (*Neophema chrysogaster*) (Stojanovic et al. 2021), Japanese macaques (*Macaca fuscata*) (Kamaluddin et al. 2019), various mammals (Siciliano-Martina et al. 2021a). Adaptation to captivity during ex situ breeding may reduce the suitability of animals in ex situ populations for conservation interventions, such as reintroductions, with captive animals performing suboptimally compared to their wild counterparts (Williams & Hoffman 2009). In the Mallorcan midwife toad (*Alytes muletensis*), prolonged breeding in captivity (more than 9 generations) resulted in reduced expression of anti-predator defences in tadpoles following exposure to predator cues (Kraaijeveld-Smit et al. 2006). As well as impacting the performance of the released animals, the release of poorly adapted captive-bred individuals can have a negative effect on the wild population (Lynch & O’Hely 2001; Ford 2002; Champagnon et al. 2012).

Adaptation to captivity is most often examined from the perspective of genetic change during ex situ breeding programmes. In response to this, a range of techniques have been proposed to mitigate genetic adaptation to captivity, such as minimizing selection and minimizing the number of generations in captivity (Frankham et al. 2002; Frankham 2008; Williams & Hoffman 2009). These methods can all influence the extent of adaptation to captivity. Equalization of family size reduces reproductive variance between families, reducing selection (Allendorf 1993; Frankham 2008; Williams & Hoffman 2009). In Drosophila (*Drosophila melanogaster*), populations with equalization of family size showed less genetic adaptation to captivity and had improved reproductive fitness (Frankham et al. 2000). However, there was no significant improvement in fitness in simulated 'wild' conditions. Equalization of family sizes may not be practical in all species, especially those with long generation time and low fecundity, where simply maximizing population size may be more appropriate (Zheng et al. 2005). In these cases, minimizing mean kinship (Ballou & Lacy 1995) may be more suitable, with pairings chosen based on kinship, rather than those that reproduce well in captivity. Minimizing kinship has a similar effect to equalizing family sizes (Frankham 2008), and can preserve genetic diversity and reduce inbreeding (Saura et al. 2008). As such, mean kinship strategies are often recommended for species in conservation breeding programmes (Cronin et al. 2006; Williams & Hoffman 2009). Creating captive environments that mimic wild environments has also been suggested to reduce inadvertent selection during ex situ breeding programmes (Frankham 2008; Williams & Hoffman 2009). In the Socorro isopod (*Thermosphaeroma thermophilum*), populations with habitat structures more similar to those in the wild showed reduced rates of change compared to those with more disparate habitats (Shuster et al. 2005). However, whilst certain accommodations can be made, it is difficult to replicate the wild environment in its entirety due to a lack of predators, reduced disease etc. (Frankham 2008). Reducing generational time in captivity reduces the opportunity for adaptation. In the Mallorcan midwife toad, although long-term captive populations showed reduced expression of anti-predator defences, those in captivity for a shorter period (3-8 generations) maintained anti-predator responses (Kraaijeveld-Smit et al. 2006). However, many species have already been maintained in ex situ populations for a large number of generations, meaning this method is not always feasible in practice. In line with these methods, a number of species management plans have been set up in ex situ breeding programmes, which aim to maintain genetic diversity and minimize inbreeding in captive populations, such as the EAZA Ex Situ Programme (EEP) (EAZA 2022a) and the AZA Species Survival Plan (SSP) (AZA 2022).

1.5.2 Behavioural adaptation to captivity

As well as genetic change during ex situ breeding programmes, animals may show behavioural changes and adaptation to the captive environment, which could significantly impact conservation programmes. Behavioural adaptation is generally less rigorously managed than genetic adaptation and, as a result, may be overlooked in some cases. Monitoring of these behavioural traits is an important first step to understand the role they play in conservation programmes (McDougall et al. 2006). This is particularly relevant for behaviours that are learned or culturally transmitted, as genetic management is unlikely to guard against the loss of these traits (Snyder et al. 1996). Social learning is an important factor in a range of behaviours important for survival, and therefore conservation, including foraging, movement, and communication (Brakes et al. 2021). As such, encouraging the performance and maintenance of these and other species-typical behaviours is important in ex situ breeding programmes (Greggor et al. 2018). In addition to learning, behavioural adaptation to captivity is also likely to be affected by interactions between animals' environment and genotype (Håkansson & Jensen 2005). For example, inadvertent selection for 'tameness' in captive breeding programmes may result in changes in animals' temperament, which, in turn, can affect a suite of conservation-relevant behaviours such as foraging, anti-predator, and social behaviours (McDougall et al. 2006).

Behavioural change during captive breeding has been well studied in red junglefowl (*Gallus gallus*) (Håkansson & Jensen 2005, 2008; Håkansson et al. 2007). Captive junglefowl varied across a range of conservation-relevant behaviours. Different populations of captive jungle fowl showed clear differences in antipredator, sexual, and exploratory behaviours (Håkansson & Jensen 2005). Although it was not possible to compare these populations to wild-type birds, the authors suggest that differences between populations could reflect adaptation to differing captive environments (Håkansson & Jensen 2005). Furthermore, behavioural differences in fear responses persisted when offspring from different captive populations were raised in a common environment, indicating that there may be a genetic component to some of the behavioural differences observed mediated by inadvertent selection during captive breeding (Håkansson et al. 2007). Social behaviour did not differ between birds of different origin, suggesting that this may be more heavily influenced by the current social environment (Håkansson et al. 2007). A further study examined antipredator responses following three generations of hatching and rearing in the same environment (Håkansson & Jensen 2008). Antipredator behaviour still showed population-specific differences. However, behavioural responses changed over multiple generations, confirming that the captive environment influenced behavioural change, as suggested in the earlier study. In addition, responses of individuals from different starting populations became more similar over successive generations, highlighting the potential influence of the current captive environment (Håkansson & Jensen 2008). Across the studies, the behavioural differences reported may be relevant for conservation programmes, as these behaviours are essential for survival in situ (Håkansson & Jensen 2005, 2008; Håkansson et al. 2007).

1.6 Conservation behaviour

The importance of considering behaviour in conservation has long been established (Beissinger 1997; Sutherland 1998). There are a number of areas where animal behaviour research could make a valuable contribution to conservation, including the role of mating systems in inbreeding depression, information of reproductive behaviour and physiology, the success of captive breeding programmes, and the maintenance of cultural skills during interventions (Sutherland 1998). A number of behaviours can reduce the effective population size (N_e), particularly those relating to reproduction and mate selection (Anthony & Blumstein 2000), and this is an important consideration in the management of small populations of conservation concern. Whilst attention to behaviour in conservation has increased, often, it is still considered to be underutilized (Angeloni et al. 2008; Nelson 2014; Berger-Tal et al. 2016). In 2014, it was reported that ~1% of behaviour papers mentioned conservation and ~5% of conservation papers mentioned behaviour across leading journals of the fields (Nelson 2014).

Across behaviours, research interest is skewed towards certain categories (Berger-Tal et al. 2016). Dispersal and foraging behaviours are among the most well studied, whereas personality, learning, and social behaviours ranked at the lower end of the spectrum (Berger-Tal et al. 2016). Despite this, the incorporation of learning and social behaviours into conservation has been successful in many cases. In the California Condor (*Gymnogyps californianus*) Recovery Programme, puppet reared birds were initially raised in cohorts without adult mentors (Walters et al. 2010). However, these birds displayed undesirable behaviour and lacked social skills and wariness of humans (Walters et al. 2010). The inclusion of interactions with adult mentors, along with further opportunities to learn through interactions with free-living birds, has results in puppet-rearing producing birds with appropriate behavioural repertoires that have similar post-release survival to parent-reared birds (Walters et al. 2010). Similarly, learning has played a key role in release programmes for migratory birds; teaching migratory routes through the use of ultralight aircraft has been an important component in reintroductions of the sandhill crane (*Grus canadensis*) (Lishman et al. 1997) and whooping crane (*Grus americana)* (Mueller et al. 2013).

There is, therefore, a need to further examine the roles of behaviour and behavioural change in ex situ conservation breeding programmes and how these could impact on the success of conservation management (Greggor et al. 2016). In particular, behaviours that may play a role in reducing N_e, such as those involved in mate selection and reproduction, as well as learned behaviours that are necessary for the performance of species-appropriate behaviour and responses, seem to be particularly relevant for conservation programmes.

1.7 Vocal behaviour

Acoustic communication is an important feature of bird biology. Vocalizations allow large amounts of information to be transmitted rapidly and efficiently (Catchpole & Slater 2008). Bird vocalizations are generally categorized into songs and calls (Catchpole & Slater 2008; Lovette & Fitzpatrick 2016). Calls are shorter, simpler vocal signals, and are produced throughout the year by both sexes (Catchpole & Slater 2008). Calls are typically used in specific situations, such as flock cohesion, alarm, and parent-offspring communication (Catchpole & Slater, 2008; Lovette & Fitzpatrick, 2016). Songs, on the other hand, are longer, more complex vocalizations, often produced by males in the breeding season (Catchpole & Slater 2008). However, whilst songs have typically been studied in male birds, there is significant evidence that female song is both important and commonplace in many species (Garamszegi et al. 2007; Odom et al. 2014). There is some overlap between simple songs and complex calls, but these distinctions are still considered useful (Catchpole & Slater 2008). Songs are typically performed by the oscines, which were separated taxonomically due to their complex syringeal muscles, and are often considered 'true songbirds' (Catchpole & Slater 2008).

Vocal behaviour, particularly birdsong, has a number of roles important roles in avian communication systems, such as territory defence and mate selection. These roles are especially important for conservation programmes, as they can affect the formation and maintenance of pair bonds, breeding patterns, and potentially gene flow within and between populations (Lewis et al. 2021). The importance of these roles in a conservation context is discussed in detail in Chapter 2.

As vocal behaviour is a conspicuous signal, it is often used as a tool during in situ conservation programmes. Due to recent advances in recording technologies and analysis, such as the development of autonomous recording units (ARUs), it has become possible to collect large amounts of acoustic data with comparatively little effort (Brandes 2008; Teixeira et al. 2019). These applications are discussed further in Chapter 2, but include species and biodiversity monitoring, and assessing environmental change (Brandes 2008; Potamitis et al. 2014; Teixeira et al. 2019; Lewis et al. 2021). However, whilst vocal behaviour is well used as a tool in conservation programmes, how vocal behaviour can affect the success of conservation programmes is less well known (Lewis et al. 2021).

1.7.1 Vocal learning

In the oscine passerines, vocal learning is an essential component of normal song development (Beecher & Brenowitz 2005). Birds that are not exposed to a tutor song during the sensitive phases of song development can develop atypical vocalizations (Price 1979; Marler & Sherman 1985; Chaiken et al. 1993; Feher et al. 2009; Kagawa et al. 2014). However, this is not the case in all species; some birds have been found to produce normal songs when reared in acoustic isolation (Kroodsma et al. 1997; Leitner et al. 2002). In addition, those tutored by heterospecifics may incorporate song features of the tutor species in their vocalizations (Johannessen et al. 2006; Eriksen et al. 2009; Mann et al. 2021). The period in which song learning can occur differs widely between species, from a brief period early in life to the whole lifetime (Beecher & Brenowitz 2005). Species in which birds continue to expand their vocal repertoire after the first year are known as open-ended learners, whereas those that do not are known as closed-ended learners (Beecher & Brenowitz 2005; Catchpole & Slater 2008). Birds also vary in the number of songs they learn, the accuracy of learning (copying fidelity), the role of early experiences, and the degree of canalization of species—specific parameters (Beecher & Brenowitz 2005).

Whilst vocal learning is most often examined in the oscine passerines, there is also evidence that other bird taxa, specifically parrots and hummingbirds, exhibit vocal learning (Catchpole & Slater 2008). As such, in these three groups, learning is necessary for the performance of species-appropriate vocal behaviours, which could prove important during conservation programmes.

1.8 Variation in vocal behaviour

Variation in vocal behaviour among species is well-documented (Catchpole & Slater 2008), and plays an important role in conspecific identification and recognition (e.g., Clayton 1990) However, vocal behaviour also varies at smaller scales within and between populations.

1.8.1 Individual level variation

Within populations, variation at the individual level is common. Bird song comprises a number of traits which vary between individuals, and these can form the basis for sexual selection (Gil & Gahr 2002). Singing involves complex musculature and is energetically costly (Eberhardt 1994; Suthers 1994; Gil & Gahr 2002), requires complex neural architecture (Gil & Gahr 2002; Nottebohm 2005), and may be subject to physical and developmental constraints (Nowicki et al. 1998, 2002a; Nowicki & Searcy 2005). Therefore, birdsong may be an honest signal of male quality that is relevant to female mate choice. In line with this, a number of facets of song reveal information about the signaller and are preferred by females.

Song phenotype can indicate the current condition of the signaller. In swallows (*Hirundo rustica*), song output was significantly affected by the presence of parasites (Moller 1991). Similarly, song rate in collared flycatchers (*Ficedula albicollis*) decreased following an immune challenge (Garamszegi et al. 2004). Therefore, male quality, in this case related to a lack of immune challenge, is signalled through vocal behaviour and this information could be used by females during mate selection. As well as the immune status of individuals, song phenotype can reveal information about the signaller's age (Kipper & Kiefer 2010). Age may be an honest signal of quality, as older males have proved their quality through longevity (Kokko 1998; Kipper & Kiefer 2010). Many song features show delayed maturation, with significant changes in song parameters between 1-year-old and 2-year-old birds (Kipper & Kiefer 2010). In common nightingales (*Luscinia megarhynchos*), first year birds had significantly smaller repertoires than older birds (Kiefer et al. 2006), but there was no evidence for longitudinal change in song of birds 2-years-old and above (Kipper et al. 2004). In some species, there is evidence for change after the second year (Ballentine 2009; De Kort et al. 2009; Vehrencamp et al. 2013; Kochvar et al. 2022). In banded wrens, older individuals have increased trill consistency compared to younger birds, which may be achieved through practice (De Kort et al. 2009). In support of the relevance of increased consistency in signalling quality, banded wrens discriminated between songs from the same individual in its first and third breeding season, and those with artificially enhanced consistency (De Kort et al. 2009). It is possible that birds may eventually display behavioural senescence of song traits. Male song sparrows (*Melospiza melodia*) showed delayed maturation of some song characteristics between year 1 and year 2, but showed declines in song rate, stereotypy within songs, and consistency between songs later in life (Zipple et al. 2019).

Vocal behaviour can also provide information on the signaller's genetic background. In canaries (*Serinus canaria*), genetic differences influence hearing, learning biases, and the proportions of low- and high-pitched syllables within songs (Wright et al. 2004; Mundinger 2010; Mundinger & Lahti 2014). In zebra finches (*Taeniopygia guttata*), a number of song traits, such as song duration, frequency modulation and amplitude, had a heritable component (Forstmeier et al. 2009) and may, therefore, provide information on the genetic background of individuals. However, many of these traits still have significant learned components, as demonstrated by cross-fostering experiments (Clayton 1990), Song traits, particularly those related to timbre of vocalizations were genetically correlated with body size, suggesting they may be an honest index signal of size, perhaps through correlations with the length of the vocal tract (Forstmeier et al. 2009).

Song phenotype also reveals information on the developmental history of the singer, since the developmental environment plays a key role in song development. The developmental stress hypothesis postulates that stress encountered early in life can influence song development, and these influences persist into adulthood (Nowicki et al. 1998; Nowicki & Searcy 2005). As such, the honesty of bird song would be maintained by developmental costs (MacDougall-Shackleton & Spencer 2012). Young songbirds are susceptible to nutritional stress during early development, resulting in a potential trade-off between investing in the song system and other developmental aspects (Nowicki et al. 1998, 2002a; Nowicki & Searcy 2005). In line with this, swamp sparrows (*Melospiza georgiana*) with poor early nutrition produced significantly less accurate copies of their song models and had significantly smaller song system nuclei (Nowicki et al. 2002a). In zebra finches, on the other hand, nutritional stress did not affect learning accuracy, but birds with parents on restricted diets had reduced song complexity compared to unrestricted birds (Zann & Cash 2008). Poor nutrition early in life in zebra finches resulted in markedly reduced antioxidant defences in adulthood (Blount et al. 2003). As such, if songs indicate nutritional stress, this may provide information on other aspects of an individuals' quality. Developmental stress may also result from increased sibling competition, with birds from larger broods experiencing greater stress. In Bengalese finches (*Lonchura striata domestica*), birds from larger, male-biased broods, which likely experience greater levels of developmental stress, had lower song complexity than those from smaller broods (Soma et al. 2006). In zebra finches, birds from large broods had less accurate song syntax learning and lower consistency of motif duration than those from smaller broods (Holveck et al. 2008). Parasite infection early in life can also affect adult song phenotype; male canaries infected with malaria early in life produced simpler songs as adults than their uninfected peers (Spencer et al. 2005).

In line with these features representing honest indicators, they are involved in mate choice and reproductive fitness. Females often show preferences for energetically challenging songs, such as those with long durations or fast tempos (Nolan & Hill 2004; Dunning et al. 2020). Females also show preferences for vocal consistency, the degree to which spectral properties of song elements are produced consistently from rendition to rendition (Botero & de Kort 2011; Sakata & Vehrencamp 2012). As discussed above, this measure is affected by age (Kao & Brainard 2006; De Kort et al. 2009; Rivera-Gutierrez et al. 2011) and developmental stress (Holveck et al. 2008) suggesting its role as an honest indicator. As such, females show preferences for consistency; in zebra finches, females preferred higher-consistency directed over undirected songs (Woolley & Doupe 2008). Increased song consistency is also related to males' reproductive fitness (Byers 2007; Cramer et al. 2011). In the chestnut-sided warbler (*Setophaga pensylvanica*), males singing with little variability had higher extrapair reproductive success (Byers 2007).

Song complexity, both in the form of large repertoires and versatility may also influence mate choice (Collins 2004). Complex songs may incur neural costs during development, and therefore indicate male quality, supported by the influence of developmental stress on complexity in some species (Soma et al. 2006). However, the effect of complexity on reproductive success is still debated (Soma & Garamszegi 2011). A meta-analysis revealed a small but significant link between song complexity and reproductive success, but this may have been overestimated due to publication biases (Soma & Garamszegi 2011). Effects of complexity were lower when complexity was measured by song versatility and when considering genetic, rather than social, mating success. Although overall effects may be small, there is still evidence that song complexity is preferred by females in a number of species (Leitão et al. 2006).

Females may also prefer males that are 'good learners', as reduced learning accuracy may signal developmental stress (Collins 2004). Females respond more to learned songs than those of males reared in isolating, highlighting the significance of learning in response (Searcy et al. 1985). In song sparrows, experienced females preferred males with a high proportion of learned, rather than improvised, content and high copy accuracy (Nowicki et al. 2002b).

1.8.2 Population level variation

Vocal behaviour can also vary between populations. A number of factors can contribute to population differences in vocal phenotype. Exposure to pollution is correlated with song phenotype, although causal links have not been established (Gorissen et al. 2005; Hallinger et al. 2010). Great tits (*Parus major*) exposed to heavy metal pollution had smaller repertoires and sang less than those in control areas (Hallinger et al. 2010). Song phenotype may also be affected by other, sympatric species. In white-rumped munia (*Lonchura striata*), song complexity was related to the number of closely-related sympatric species, suggesting that complexity was constrained to aid conspecific recognition (Kagawa et al. 2012).

Population-specific dialects, cultural variation between populations as a result of vocal learning, are well-studied and widely reported (Podos & Warren 2007; Catchpole & Slater 2008; Lovette & Fitzpatrick 2016). These dialects represent clusters of similar vocalizations that are different from other clusters, and can be measured using a range of vocal features. For example, in the yellow cardinal (*Gubernatrix cristata*), dialects are described by a number of features, including emphasized frequency, number of notes and proportion of simple notes, which differ consistently between north and south populations (Domínguez et al. 2016). Dialects can form via a range of mechanisms, which are discussed in more detail in Chapter 2. Briefly, dialect formation can be influenced by cultural processes, such as cultural drift (Mundinger 1980), withdrawal of learning (Thielcke 1973) and cultural bottlenecks (Baker & Jenkins 1987; Hill et al. 2013), acoustic adaptation to habitats (Boncoraglio & Saino 2007), the avoidance of anthropogenic noise (Slabbekoorn et al. 2003; Brumm & Slabbekoorn 2005; Nemeth & Brumm 2009; Luther & Baptista 2010; Cardoso & Atwell 2011), and the avoidance of biotic noise from other species (Brumm & Slabbekoorn 2005; Luther 2009; Grant & Grant 2010).

Dialects may influence mate choice, with females preferring local over foreign dialect songs (Baker 1983; MacDougall-Shackleton & MacDougall-Shackleton 2001; Searcy et al. 2002; Hernandez et al. 2009). Preferences based on dialect can promote assortative mating, where animals select mates based on phenotypic similarity to themselves (Jiang et al. 2013). Whilst there is some suggestion that dialect-based assortative mating could be adaptive (Slabbekoorn & Smith 2002; Nowicki &

Searcy 2005; Podos & Warren 2007; Snowberg & Benkman 2007) preference for local dialects may be solely related to stimulus familiarity (Slabbekoorn & Smith 2002), which is supported by a lack of preference in females from mixed-dialect populations (Chilton et al. 1990, 1996). In this way, geographic divergence in vocalizations can facilitate assortative mating and reproductive isolation. Irwin et al., (2001) used song playback to investigate reproductive isolation in the greenish warbler (*Phylloscopus trochilodes*), a species where songs vary clinally in a ring. They used responses by males to playbacks to determine whether different groups viewed each other as potential mates or competitors. Along the cline, males respond to playback from recordings taken 1000-1500km away but no further. Where the two terminal subspecies, which exhibited large differences in dialect, were compared, there was virtually no response regardless of distance between the male and the recording, suggesting that song variation could contribute to pre-mating isolation. However, the exact role of song in reproductive isolation and speciation likely depends on a range of ecological factors and life history traits, such as song learning, dispersal, and habitat (Slabbekoorn & Smith 2002; Podos & Warren 2007). The roles of dialects in territory formation and maintenance, mate choice, and gene flow are further discussed in Chapter 2.

1.9 Vocal behaviour in captivity

Differences in vocal behaviour between wild and captive populations have been reported in a number of avian and non-avian taxa (Passos et al. 2017; Tanimoto et al. 2017; Martínez & Logue 2020; Crates et al. 2021). Comparisons between the past wild and current populations of the Hawaiian crow (alala: *Corvus hawaiiensis*) found that, although acoustic richness and diversity of calls were similar, repertoires differed significantly (Tanimoto et al. 2017). Captive birds produced fewer alarm calls and did not produce territorial broadcast calls. Regent honeyeaters (*Anthochaera phrygia*) raised in captivity had markedly reduced song complexity compared to wild-origin birds (Crates et al. 2021).

Many of the features that may drive vocal change and divergence in ex situ environments are comparable to those involving the formation of population-specific dialects, discussed above and in Chapter 2. However, some of these mechanisms may have particular relevance to ex situ breeding programmes, which are summarized below.

The influence of cultural processes, such as drift, on song may differ for populations in ex situ breeding programmes compared to those in situ. Populations in ex situ breeding programmes are often relatively small and separate. Cultural drift may be more intense in small, fragmented populations (Laiolo & Tella 2007). In mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*), song types and syllables changed more in small or more fragmented populations compared to large, continuous populations (Harbison et al. 1999). There is also the potential for repeated cultural bottlenecks as animals are moved between locations, which can influence song structure and diversity (Laiolo & Tella 2007; Laiolo 2010; Parker et al. 2010, 2012). In the North Island saddleback (*Philesturnus rufaster*), serial translocations resulted in a reduction of song type sharing with the ancestral population (Parker et al. 2012). Vocal behaviour in captive environments may also be influenced by a reduction in learning opportunities during ex situ breeding programmes. As discussed, regent honeyeaters raised in captivity had lower complexity songs than birds of wild origin (Crates et al. 2021). Captive juveniles are typically housed away from adults, meaning they have reduced opportunities to associate with and learn from adult conspecifics, which may contribute to the unique song culture in captivity (Crates et al. 2021). Vocal behaviour in captive birds may also be influenced by a lack of appropriate stimuli. For example, the reduced alarm calls and lack of territorial broadcast calls in the Hawaiian crow may result from a lack of appropriate stimuli and opportunities to produce them (Tanimoto et al. 2017).

Birds in captive environments are likely to experience significantly different sound environments to those in the wild. There is likely to be significant variation between different captive animal facilities, but general sound sources are likely to be similar across situations (Clark & Dunn 2022). Broadly, these can be categorized as permanent sources of mechanical noise, such as heating, ventilation, and air conditioning (HVAC systems), temporary noise from equipment used for maintenance and communications, human vocalizations and footfall, and sounds produced by animals in the facility (Clark & Dunn 2022). Enclosures can affect the properties of sound and how animals are able to respond (Hughes 2007; Schneider & Dierkes 2021; Clark & Dunn 2022). For example, animals may have to contend with reverberation, particularly in enclosures with hard, smooth surfaces (Hughes 2007; Schneider & Dierkes 2021), which are commonplace in captive environments (Clark & Dunn 2022). In zebra fish (*Danio rerio*), natural soundscapes had lower, more variable, sound pressure levels than captive soundscapes and the frequency bands in which sound energy was concentrated varied between environments (Lara & Vasconcelos 2019). An examination of the sound environment in Zoo de Granby, Canada, found that sound intensity and patterns of variation differed between locations within the zoo, suggesting species in different locations may be differentially affected by the sound environment (Pelletier et al. 2020). Significant anthropogenic noise in zoos may result in similar responses to birds in urban environments, such as increased amplitude and frequency of vocalizations (Slabbekoorn et al. 2003; Luther & Baptista 2009; Nemeth & Brumm 2009), and contribute to vocal change in the captive environment. As well as adaptation to different sound environments, captive environments are necessarily different to those in situ, which may promote song divergence from wild-type vocalizations through acoustic adaptation to captive habitats.

Although not specifically related to vocal learning, vocalizations of birds in ex situ breeding programmes may also be affected by other forms of adaptation to captivity. Morphological differences between wild and captive populations are reported across a broad range of taxa (mammals (e.g., Kamaluddin et al., 2019; Pelletier et al., 2021; Siciliano-Martina et al., 2021b, 2021a), birds (e.g., Remeš & Székely, 2010; Soma, 2005; Stojanovic et al., 2021), amphibians (e.g., Kraaijeveld-Smit et al., 2006), fish (e.g., Bernatchez et al., 2008; Pulcini et al., 2013)). In birds, morphology plays a role in vocal behaviour, with beak morphology and body size influence the production of vocalizations (Podos 2001; Huber & Podos 2006; Derryberry et al. 2012, 2018; García & Tubaro 2018; Friedman et al. 2019). In general, larger body size is associated with lower vocal frequencies and whereas larger beaks are associated with produce songs with slower tempo of vocalizations (Derryberry et al. 2012, 2018; García & Tubaro 2018; Friedman et al. 2019). As such, morphological change during captive breeding programmes could contribute to divergence in vocalizations.

1.10 The influence of vocal behaviour on conservation programmes

As mentioned previously, behaviours that may be particularly relevant in conservation programmes are those which can act to reduce the effective population size (N_e), such as those involved in mate choice, and learned behaviours necessary for the performance of species-appropriate behaviour and responses. Vocal behaviour fits into both of these categories, suggesting it may influence conservation efforts and is important to investigate further. The role of vocalizations across a range of conservation strategies is discussed in depth in Chapter 2 and summarized below.

Preferences for familiar vocalizations during conservation programmes may influence mate choice and therefore reproductive success. If vocal behaviour changes significantly during ex situ breeding, animals may not recognise and respond appropriately to individuals from different populations in conservation programmes. Although the extent to which vocal behaviour can result in reproductive divergence and speciation requires further investigation (Slabbekoorn & Smith 2002), even small differences in mate choice and assortative mating could be important in conservation programmes. Increased selectivity increases the probability of mate rejection which may result in fewer individuals finding suitable mates and contribute to population declines (Bessa-Gomes et al. 2003). The effect of vocal change during captive breeding on reproductive behaviour has been examined in golden mantella frogs (*Mantella aurantiaca*). Calls of captive golden mantella were significantly different to those of wild frogs in a range of temporal and frequency-based features (Passos et al. 2017). Furthermore, zoo-bred golden mantella showed a strong response to playback of calls from their own population, but only a weak response to calls of wild origin. Despite this, wild origin frogs responded similarly to all playback calls (Passos et al. 2017). As such, captive-bred frogs may behave sub-optimally during reintroduction efforts, reducing the success of interventions.

1.11 The Java Sparrow (Lonchura oryzivora)

In this thesis, I focus on a single species, the Java sparrow (Figure 1.1), as a model for examining the potential for vocal change during captive breeding and the effect this may have on conservation efforts.

1.11.1 General information

Java sparrows are native to Indonesia and found as an invasive species across a range of countries in South East Asia and beyond (Restall 1996; BirdLife International 2018b). The species is also frequently found in zoological collections worldwide and is a popular bird in aviculture. This species is associated with a wide range of wild and human-dominated environments, and is frequently associated with cultivated land growing cereal crops, such as rice (Restall 1996; BirdLife International 2018b). Birds have monotypic plumage, with both males and females displaying the same predominantly grey plumage, black head, white cheeks, and pink beak (Figure 1.1; Restall 1996). Male and female Java sparrows engage in duet dancing during courtship, with males also producing vocalizations (Soma & Iwama 2017). Both sexes are involved in nest-building and parental care of offspring in the nest (Restall 1996).

Figure 1.1: The Java sparrow (*Lonchura oryzivora*) © Chester Zoo

1.11.2 Conservation status

In general, Asian songbirds are a group of key concern for conservation efforts. Although Asian songbirds are less threatened overall than birds in general, they are declining at an alarming rate (BirdLife International 2018a). High levels of offtake for many South-East Asian bird species to fuel the cage-bird trade has perpetuated the 'Asian Songbird Crisis' (Harris et al. 2016; Lee et al. 2016; Marshall et al. 2020). In 2018, the Java sparrow was classified as 'Endangered' in the IUCN Red List (BirdLife International 2018b), having previously been classified as 'Vulnerable' (BirdLife International 2018b). Java sparrows were listed as one of the top 28 priority species at the first Asian Songbird Trade Crisis Summit held in 2015 (Lee et al. 2016) and a species-specific action plan was developed. In addition, one of the key themes of work identified during the summit was captive breeding and husbandry (Lee et al. 2016). The plight of Asian songbirds is also well recognised by organizations focused on ex situ breeding programmes. For example, EAZA (The European Association for Zoos and Aquaria) ran the Silent Forest campaign from 2017-2019, which aimed to raise awareness and supporting research initiatives both within the EAZA region and in the birds' natural range (EAZA 2022b). Java sparrows were also listed as a 'Focus species' in the campaign (EAZA 2022c). As such, Java sparrows are already a target for conservation efforts, and the need for effective conservation strategies is likely to increase as the population is currently declining (BirdLife International 2018b). Given the prevalence of the Java sparrow in zoological collections, ex situ breeding programmes play an important role in conservation efforts. This makes them an ideal model species for investigating the evolution of mating traits during ex situ conservation and exploring how this might affect their conservation.

1.11.3 Vocal behaviour

Whilst both males and females vocalize in this species, only the males exhibit singing behaviour. As in other estrildid finch species, songs are used during courtship displays, and birds are not territorial (Restall 1996). However, recent studies in the closely related zebra finch suggest that songs may also have a role in maintaining social cohesion and synchronization of breeding (Loning et al., 2023), so Java sparrow songs may also have functionality outside of courtship. Males learn to sing a single song during a critical period in the nest and songs tend to consist of 2-8 distinct note types (Ota & Soma 2014). Song learning requires social interactions, and, as such, most juveniles, particularly in a laboratory setting, learn for their social fathers if they do not interact with other adult males (Soma 2011). As vocalizations are only used in the context of courtship, the Java sparrow is a good candidate for studying the evolution of mating traits during ex situ breeding and its impacts, as songs are unlikely to be influenced by other social interactions. The Java sparrow is closely related to two species often used as models for the study of birdsong, the zebra finch and the Bengalese finch. Therefore, we can use knowledge obtained in these two species to examine how generalizable patterns are across the group and in a species with greater conservation requirements.

1.12 Journal format

This thesis is submitted in the journal (alternative) format in full accordance with the regulations stipulated by the University of Manchester. This thesis is presented as a series of chapters formatted for publication in scientific journals. Two of these chapters have already been published (Chapters 2 & 3) (see 'Published Work' (1.14)). As such, chapters may differ slightly in format and referencing style due to the requirements of the journals in which they have been published. Unpublished chapters follow a similar, generic, journal format. Presentation of chapters as stand-alone articles may result in some overlap between chapters, particularly in the 'Methods' sections where papers share datasets.

Although journal papers are a collaborative endeavour, the work presented in this thesis is predominantly my own. At all stages of the process, I was supported by my supervisory team and co-authors. Individual chapters contain a list of author contributions detailing the input of each author. For published chapters (Chapters 2 and 3), the final version includes feedback provided by editors and reviewers during the publication process. Throughout the journal-style chapters, I use 'we' as opposed to 'I' to represent the collaborative nature of scientific projects and to ensure consistency between chapters which have already been published/submitted and those which have not. A full overview of the chapters presented in this thesis is provided in the 'Thesis Overview' section below (1.13).

1.13 Thesis overview

This thesis aims to explore how the evolution of traits relevant to mating and reproductive success during ex situ breeding programmes could influence conservation programmes, with an emphasis on vocal behaviour. I focus on a single species, the Java sparrow, as a model to create a body of work and recommendations that can also be applied to other species in ex situ breeding programmes.

In Chapter 2, I present a literature review examining the role of vocal behaviour in conservation efforts. Recently, there has been an increase in the use of conservation technology, such as automated recording devices, for wildlife monitoring in conservation. However, the implications of vocal behaviour for the success of conservation programmes are less well explored. As well as further highlighting the uses of vocal behaviour as a tool in conservation, I explore how vocal behaviour could influence the success of a number of conservation intervention.

Given the potential influences of vocal learning on conservation programmes identified in Chapter 2, in Chapters 3 and 4 I examine the patterns of inheritance for a range of song features. Understanding song inheritance in the Java sparrow can help us to better determine how vocalizations may change during ex situ breeding, which is essential for incorporating vocal behaviour into conservation efforts. Both of these chapters use an archival dataset consisting of recordings from multiple generations of Java sparrows in a laboratory population. Among these birds, some were raised by their genetic father, but others were cross-fostered and raised by a social father which was not genetically related to them. This dataset, therefore, provides an excellent opportunity to examine the contributions of social learning and genetic inheritance to song development. In Chapter 3, I examine the inheritance of three major axes of song variation, which are frequently examined in the literature; song structure and complexity, individual note parameters, and note consistency. In Chapter 4, I examine the inheritance of temporal song features in Java sparrows. Inheritance of these features has received less attention across the literature. Since the inheritance of temporal features is not well understood, Chapter 4 used the dataset described above to determine if these features showed similar patterns to the more conventionally examined song features.

Once the inheritance patterns of vocalizations are understood, it is important to determine how songs could influence mating patterns. In vocal learners, like the Java sparrow, learning can result in the formation of population specific song dialects, with individual populations exhibiting their own, distinct, songs. Females' preference for familiar songs could create a pre-mating barrier to reproduction and contribute to assortative mating based on population of origin. In ex situ breeding programmes, this could reduce reproductive success when birds are moved between collections for breeding. Assortative mating based on song familiarity could also reduce the success of reintroduction programmes, as released individuals may not fully integrate and breed with other populations due to song differences. In Chapter 5, I examine the effect of song familiarity on female song preference in the Java sparrow. Using archival recordings, females were presented with paired familiar and unfamiliar stimuli, with familiar stimuli being their father's song or a song of the same type as their father. Preference was determined using behavioural responses, and behaviours were selected based on their relevance to mate choice.

Ex situ breeding programmes could result in changes in other traits, which are also relevant for reproductive success. One such trait is morphology; reduction in selection pressures, combined with artificial selection (direct or inadvertent), could result in morphological change during captive breeding. As well as directly influencing mating success, morphological change can also result in corresponding changes in vocal behaviour. In Chapter 6, I examine the evolution of size and sexual dimorphism in Java sparrow populations during ex situ breeding by comparing the size of museum specimens, birds from a zoological collection, and birds from an avicultural, laboratory population.

Finally, it is important to understand the possible drivers of vocal change in ex situ environments. One potential driver of vocal change in captivity is the different soundscape experienced by birds in captive environments. In Chapter 7, I examine the effects of the COVID-19 lockdown on the soundscape in zoo aviaries. As zoos are open throughout the year, it is unusual to have a control period without visitor presence and with reduced anthropogenic activity. Therefore, lockdown provided an interesting opportunity to explore how chronic human presence affects zoo soundscapes by examining differences between pre-lockdown and lockdown conditions.

1.14 Published work

Chapter 2
Lewis, R. N., Williams, L. J., & Gilman, R. T. (2021). The uses and implications of avian vocalizations for conservation planning. *Conservation Biology*, *35*(1), 50-63

Chapter 3
Published as part of a special issue of Frontiers in Psychology: Songs and Signs: Interdisciplinary Perspectives on Cultural Transmission and Inheritance in Human and Non-human Animals
Lewis, R. N., Soma, M., De Kort, S. R., & Gilman, R. T. (2021). Like father like son: cultural and genetic contributions to song inheritance in an estrildid finch. *Frontiers in psychology*, *12*, 2030.

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Chapter 2: The uses and implications of avian vocalizations for conservation planning

Rebecca N Lewis, Leah J Williams, R Tucker Gilman

2.1 Chapter Summary

Vocal behaviour is a conspicuous signal, which means it is easily detected and measured (Teixeira et al. 2019). As such, the use of vocalizations to detect and monitor species has increased rapidly in recent years. For example, vocal behaviour can be used to determine presence/absence of otherwise hard to observe species, whilst reducing human resources requirements and reducing disturbance of sensitive species (Teixeira et al. 2019). However, whilst the use of vocal behaviour in social interactions is useful for species monitoring and inventory, it can also negatively impact conservation efforts if not properly incorporated into conservation management plans e.g., by influencing mate selection and breeding (Rowe & Bell 2007; Bradley et al. 2014). This, in turn, can have consequences for the success of conservation interventions, such as translocations and reintroductions.

As a group, birds are an ideal candidate for exploring the effects of vocal behaviour on conservation efforts. Vocal behaviour is a key component of avian communication (Catchpole & Slater 2008). In line with this, bird vocalizations have received substantial attention over time, with a strong focus on development and functions (Baker 2001; Slater 2003). As such, a considerable body of work has already been carried out to understand avian vocal behaviour. However, this research is not often framed from a conservation perspective.

In this chapter, I present a literature review examining how existing literature, which was not necessarily aimed at informing conservation or animal management, can be applied in the context of conservation programmes. Firstly, I discuss the importance of vocalizations for conservation-relevant behaviours, such as territory defence and mate selection. Then, I discuss the potential applications of vocal behaviour in conservation programmes, examining monitoring and conspecific attraction. Finally, I examine how vocal behaviour could influence the success of conservation programmes, with a focus on interventions, such as translocations and reintroductions. As part of this, I also present a range of potential mitigation methods and areas for further research to improve the incorporation of vocal behaviour into conservation planning. This theoretical understanding of how vocal behaviour could impact on conservation efforts is a useful first step in identifying where problems could occur and in developing clear research objectives to further investigate these issues to properly account for and mitigate against them.

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The work presented in this chapter represents the author accepted version of the manuscript formatted for inclusion in the thesis. An additional discussion section (2.8) has been added in order to discuss relevant research from other taxa and that has been published since the manuscript presented in this chapter.

2.2 Abstract

There is a growing recognition that animal behavior can affect wildlife conservation, but there have been few direct studies of animal behavior in conservation programmes. However, a great deal of existing behavioral research can be applied in the context of conservation. Research on avian vocalizations provides an excellent example. The conspicuous nature of the vocal behavior of birds makes it a useful tool for monitoring populations and measuring biodiversity, but the importance of vocalizations in conservation goes beyond monitoring. Geographic song variants with population-specific signatures, or dialects, can affect territory formation and mate choice. Dialects are influenced by cultural evolution and natural selection and changes can accumulate even during the timescale of conservation interventions, such as translocations, reintroductions, and ex situ breeding. Information from existing research into avian vocalizations can be used to improve conservation planning and increase the success of interventions. Vocalizations can confer a number of benefits for conservation practitioners through monitoring, providing baseline data on populations and individuals. However, the influence of cultural variation on territory formation, mate choice and gene flow should be taken into account, as cultural differences could create obstacles for conservation programmes that bring birds from multiple populations together and so reduce the success of interventions.

2.2 Introduction

A recent assessment by BirdLife International (2018) showed that around 13% of all extant bird species are globally threatened and many more are in decline. Many conservation measures have been attempted to help reverse declines; intensive interventions such as ex situ breeding, reintroductions, and translocations have proven valuable (e.g., Cade & Jones 1993; Miskelly & Powlesland 2013). When planning these types of interventions, it is important to consider how animal behaviors may affect success.

Although attention to animal behavior in conservation has increased in recent years, there is still a broad consensus that information on animal behavior is underutilized (Angeloni et al. 2008; Berger-Tal et al. 2016). Thus, future research should examine behavior and conservation in tandem. However, there is already considerable behavioral research available that has implications for conservation and can be used to guide management strategies. Vocal communication is an easily detected, conspicuous behavior and is well described for many species (Lovette & Fitzpatrick 2016). We summarized the relevant background information on variation in avian vocalizations, considered how vocalizations influence key processes of conservation interest, and explored the potential roles of vocalizations in conservation applications.

2.3 Variation in avian vocalizations

Avian vocalizations can vary in a number of ways based on temporal factors, spectral characters (Table 2.1), and the elements, syllables, and phrases vocalizations are composed of. Variation among species, often used for conspecific recognition, and the role of among-species variation in territory defence (e.g., North Island Saddlebacks [*Philesturnus rufusater*]) (Parker et al. 2010)) and mate choice (e.g., Medium Ground Finches (*Geospiza fortis*) and Cactus Finches (*G. scadens*) (Grant & Grant 1996)) are well understood (Catchpole & Slater 2008). However, variation can occur at smaller scales within and among populations.

Within populations, individual variation is common. Such variation may be affected by intrinsic factors, such as morphology: body size affects spectral characters and beak shape affects temporal factors (Derryberry et al. 2018; García & Tubaro 2018). Vocal repertoire and repertoire size can also vary widely between individuals of the same species (Krebs & Kroodsma 1980).

Among populations, cultural variation in vocalizations, known as dialect, has been reported in many species (e.g., Wright et al. 2008; Robin et al. 2011). There are a number of suggested mechanisms for dialect formation and evolution (Catchpole & Slater 2008), and understanding these is essential to predicting the possible impacts of dialects on conservation programmes.

Cultural drift describes the random accumulation of song mutations due to copying errors and improvisation (Mundinger 1980). The action of drift is more severe in smaller populations, especially in fragmented environments, which are commonplace in conservation (Laiolo & Tella 2007). Over 19 years, calls of the Yellow-naped Amazon (*Amazona auropalliata*) showed a lower degree of stability in the smaller northern population relative to the larger southern population (Wright et al. 2008). The rate of drift may also be influenced by song traits. In White-bellied Shortwings (*Sholicola albiventris*), simple songs are still similar in populations that have been separate for thousands of years, but differences in complex song clusters are apparent in populations separated for a comparably shorter time (100-150 years) (Purushotham & Robin 2016).

Table 2.1: Glossary of specialist terms pertaining to bioacoustics and song learning*

Term	**Definition**
Amplitude	volume of sound, measured as height of sound waves in vocalization
Call	short, simple vocalizations used for social cohesion, parent-offspring communication, aggression, and signalling danger
Closed-ended learner	bird in which song learning is restricted to a short period, usually the first year of life (Beecher & Brenowitz 2005)
Cultural variation	variation among populations in information or behaviors shared by individuals and acquired from conspecifics by social learning (Whitehead & Rendell 2015)
Dialect	sets of geographic vocalization variants with distinct, population-specific vocal features
Elements	smallest divisions of birdsong, also referred to as notes
Frequency	wavelength of sound; shorter wavelengths produce higher frequencies; frequency of a call can be measured as the maximum (highest frequency), minimum (lowest frequency), mean (across the song or individual elements), and peak (frequency with the highest amplitude)
Open-ended learner	bird in which song learning can occur throughout life (Beecher & Brenowitz 2005)
Phrases	series of units (usually syllables) occurring together in a particular pattern
Repertoire	full set of vocalizations that a single individual produces
Repertoire size	total number of different vocalizations an individual produces, usually measured as the number of different song types
Song	loud, long and usually complex vocalizations most often used in courtship and territory defence
Song complexity	variously, song repertoire size, note repertoire, versatility, nonlinearity and standard deviation of frequencies have been proposed as definitions of complexity and all may act as honest signals of fitness (Soma et al. 2006; Pearse et al. 2018)
Song rate	number of songs produced by an individual per unit time
Spectral factors	factors relating to the frequency of vocalizations
Syllables	building blocks of phrases, can be complex (containing multiple elements) or simple (containing only 1 or 2 elements)
Temporal factors	factors relating to the timing of vocalizations
Withdrawal of learning	rapid song innovation following colonization by founders that dispersed before song crystallization (i.e., before they produce stereotyped, adult songs) (Thielcke 1973)

* Unless otherwise stated, definitions are based on Catchpole and Slater (2008) and Lovette and Fitzpatrick (2016)

Cultural traits like song diversity can be lost during population bottlenecks, such as the colonisation of new habitats (e.g., Baker 1996) or population reductions following habitat loss or fragmentation (Laiolo & Tella 2007). Hill et al. (2013) reported reduced syllable diversity and a lower percentage of trills in the threatened Chatham Island Tui (*Prosthemadera novaeseelandiae chathamensis*) relative to its mainland counterpart (*P. n. novaeseelandiae*). However, it is often impossible to detect bottleneck events from song diversity due to the continued action of drift or withdrawal of learning (Potvin & Clegg 2015). In such cases, past bottlenecks may be evidenced by low shared syllables between populations (e.g., Lang & Barlow 1997).

The acoustic adaptation hypothesis predicts that habitat-dependent selection shapes song evolution. Different habitats have different sound transmission properties: complex vegetation causes greater attenuation, particularly of high frequency sounds, than more open habitats (Brumm & Naguib 2009). A meta-analysis of studies examining acoustic adaptation showed a small overall effect of habitat on frequency across oscine and suboscine species; lower frequencies (minimum, maximum and peak) and smaller frequency bandwidths occur in closed habitats (Boncoraglio & Saino 2007).

Biotic noise is highly variable and may be hard for birds to avoid. There are increasing examples of spectral and temporal partitioning in the acoustic signals of wild birds, both in response to other birds (Ficken et al. 1974; Planque & Slabbekoorn 2007; Luther 2009) & and other taxa (Sueur 2002; Hart et al. 2015). Grant & Grant (2010) detail song changes of *Geospiza fortis* and *G. scadens* after the arrival of *G. magnirostris* on Daphne Major in 1983. Over the study period (1983-2010) the song traits of *G. fortis* and *G. scadens* dispersed away from those of *G. magnirostris*; changes included shorter songs and increased trill rate, which could not be explained by other changes in the environment.

It is likely that dialect formation is influenced by a combination of the mechanisms mentioned above. Given the number of possible influences, it is difficult to disentangle the driving factors, and factors may change in importance over time (Potvin & Clegg 2015; Purushotham & Robin 2016).

2.3 Importance of variation in vocalizations to processes of conservation interest

Variation in vocalizations among populations can play an important role in a number processes important for population persistence, such as territory maintenance, mate choice, and gene flow.

2.3.1 Territory formation and defence

In many species, holding territories is essential for resource acquisition and the formation and maintenance of pair bonds and so improves mating success (Hinde 1956). Successful territory maintenance relies on the ability to identify conspecifics and respond by defending the territory. However, birds respond more strongly to unfamiliar songs of their own dialect and to songs that are more similar to their own than to foreign dialects (e.g., Searcy 1997). As a result, birds may fail to adequately defend their territories from conspecifics with foreign dialects. Irwin et al. (2001) examined responses of the Greenish Warbler (*Phylloscopus trochiloides*), a species where songs vary clinally in a ring. Along the cline, males respond to playback from recordings taken 1000-1500 km away but no further. When the 2 terminal subspecies, which have come into secondary contact and exhibit large differences in dialect, were tested, neither responded to the other regardless of distance between the male and the recording. The combined evidence from territory studies suggests birds may find it difficult to defend territories where foreign dialects are prevalent (Slabbekoorn & Smith 2002), which could reduce access to food, nest sites, and mates. This may cause problems if birds with different dialects are brought together during conservation interventions (Parker et al. 2010; Bradley et al. 2013; Valderrama et al. 2013).

2.3.2 Mate choice

Birdsong plays an important role in female mate choice. There is evidence that performance-related factors, such as amplitude and rate (e.g., Ballentine et al. (2004) but see Kroodsma (2017) for critique), song complexity, and repertoire size (Searcy 1992; Byers & Kroodsma 2009) may influence female preference and could act as honest signals of male quality (Gil & Gahr 2002). Dialect can also influence female mate choice. Female preference for local dialects can promote assortative mating, where animals select mates genetically or phenotypically similar to themselves (Jiang et al. 2013). Searcy et al. (2002) found that female song sparrows showed similar responses to local and nearby (18 km) foreign dialects, but discriminated against dialects from greater distances (34 km, 68 km, and 135 km).

Selectiveness in mating can lead to an increased probability of mate rejection, reducing overall mating rates. Where mating opportunities are limited, as in some small, endangered populations, this may result in fewer individuals finding mates and breeding, potentially contributing to population declines and extinction (Bessa-Gomes et al. 2003). Assortative mating plays a key role in premating reproductive isolation, possibly resulting in speciation (Kirkpatrick 2000; Verzijden et al. 2012), and can promote reproductive isolation at secondary contact (Grant & Grant 2002) – including during conservation interventions when previously separated populations are brought together. This has been reported following multiple conservation interventions in the North Island Kokako (*Callaeas wilsoni*) (Bradley et al. 2014).

Female preference for local dialects could be adaptive; females may gain fitness advantages by choosing males from their natal region (Slabbekoorn & Smith 2002; Podos & Warren 2007). Whilst singing a local dialect should not be inherently more costly than singing a foreign dialect (Nowicki & Searcy 2005), dialects could act as behavioral markers for other traits. In the Red Crossbill (*Loxia curvirostra*), song and bill morphology are strongly correlated. Mating within the local population, signaled by dialect, prevents the production of offspring with intermediate, less fit phenotypes (Snowberg & Benkman 2007). Local dialect may also signal males with local experience (Searcy 1982) who may be better able to secure resources for females and their offspring, thus providing direct benefits to choosy females.

Dialect preferences could also be a nonadaptive result of familiarity (Slabbekoorn & Smith 2002). Female White-crowned Sparrows in mixed-dialect populations show neither preference for their fathers' dialects nor consistent preference for any dialect across successive breeding seasons (Chilton et al. 1990). In captivity females do not respond differently to two dialects they commonly hear within their population, but show reduced responses to a dialect from a different population (Chilton et al. 1996). These results suggest females from mixed-dialect populations can distinguish between dialects, but show no difference in preference among songs they commonly hear. Females exhibiting a preference (for father's dialect or otherwise) would be expected to mate with birds of the same dialect across seasons. However, if preferences do not align with mate choice in the field, dialect may not have a profound effect on mating during conservation.

2.3.3 Gene flow

If dialects contribute to mate choice, they may reduce gene flow by reducing breeding between populations. Such inbreeding can affect fitness and affect both individual and population performance (Keller & Waller 2002). For example, inbreeding reduced hatching success, fledgling survival, and recruitment in the Red-cockaded Woodpecker (*Picoides borealis*) (Daniels & Walters 2000).

A number of studies report substantial genetic mixing between populations despite dialect differences (e.g., Orange-tufted Sunbirds (*Cinnyris bouvieri*), Leader et al. (2008) and Puget Sound White-crowned Sparrow (*Z. l. pugetensis*) (Poesel et al. 2017)). Other studies show some genetic structuring related to dialect differences (e.g., Mountain White-crowned Sparrows (MacDougall-Shackleton & MacDougall-Shackleton 2001)). Even small reductions in gene flow can reduce the effective population size, potentially contributing to reduced heterozygosity and Allee effects (Chesser et al. 1993).

Although dialects could limit gene flow between populations and increase genetic differentiation, other factors may also contribute. In particular, dialect differences are often related to distance between populations. In the White-bellied Shortwing, song and genetic differences appear highly correlated. However, when controlling for geographic distance and dispersal barriers, spectral and syntax differences are not correlated with genetics (Purushotham & Robin 2016).

There is little consensus on the impact of dialects on gene flow, and some studies even show conflicting results for the same species and populations (e.g., Nuttall's White-crowned Sparrow (*Z. l. nuttali*): Baker & Mewaldt 1978; Petrinovich et al. 1981; Baker et al. 1982; Zink & Barrowclough 1984; Hafner & Petersen 1985; Soha et al. 2004). This is likely due to methodological differences (e.g., marker used, scale of study, populations or subspecies chosen). The significance of dialect for gene flow is likely to vary by species due to life-history traits (e.g., dispersal rates and mating systems) and vocal learning: birds learning song before dispersal are more likely to be affected than those learning throughout life (Podos & Warren 2007). Overall, there is little evidence to suggest that dialect alone could completely prevent gene flow between populations: even a few cross-dialect pairs per generation would be sufficient to prevent divergence (Potvin et al. 2013). More information is required to provide useful evidence for conservation practitioners. Monitoring gene flow and dispersal events across a range of species, particularly endangered species during conservation interventions, should be a priority for researchers examining genetics in avian conservation.

2.4 Uses and implications of avian vocalizations for conservation

Understanding the biology of avian vocalizations and their impact on population processes can help one appreciate the role of vocal behavior during conservation. We considered potential applications for the use of avian vocalizations (summarized in Table 2.2) and the potential negative effects of variation in vocalizations and how they might be overcome (summarized in Table 2.3).

2.4.1 Monitoring

The conspicuous nature of vocalizations means they are easy to measure, even in complex environments, making them a useful non-invasive tool for monitoring (Teixeira et al. 2019). With recent advances in recording technologies and analysis, such as automated recording units, it has become possible to collect large amounts of acoustic data with comparatively little effort through passive acoustic monitoring (PAM) (Brandes 2008). Bioacoustic methods perform as well as traditional point counts in a number of cases (Alquezar & Machado 2015; Darras et al. 2018).

There remains a need to create standardized practices for acoustic detection surveys (Darras et al. 2018), but a wealth of data with conservation relevance can be collected using these methods.

Acoustic monitoring can provide useful baseline data by examining the spatial and temporal variation of sound (e.g., Pieretti et al. 2011; Rodriguez et al. 2014; Sebastian et al. 2016). Vocalizations extracted from recordings can be used for a number of purposes, including detecting species presence or absence, identifying preferred habitats, detecting juveniles, and determining predator abundance from alarm calls (Teixeira et al. 2019).

Interindividual variation within species allows conservation practitioners to improve the resolution of baseline data (Terry et al. 2005). The ability to discriminate between vocalizations of different individuals can be useful for population censuses (Terry et al. 2005). However, this requires that all birds in a given area vocalize during the sampling period or that researchers have some knowledge of the proportion of birds singing (e.g., if males sing and females do not). If not all birds vocalize, or if vocalizations are biased toward certain demographics or areas (e.g., Legare et al. 1999), population sizes may be underestimated or habitat use may be misinterpreted. The ability to identify individuals by assigning vocalizations to known birds is considerably harder but, when possible, provides useful, high-resolution data. For example, this can allow researchers to assess how life-history traits, such as survival, vary among individuals (Terry et al. 2005).

Bioacoustic data can be used to measure responses to environmental perturbations or human disturbance. Anthropogenic noise can affect birds in a number of ways, including altering habitat use and influencing the characteristics of vocal signals (Ortega 2012). Deichmann et al. (2017) used PAM to examine the impacts of natural gas exploration on avian biodiversity and found diversity increases as distance from the drilling site increases. Such information can be used to minimise the impacts of future disturbances and advise conservation programmes. Bioacoustic monitoring can also be employed to evaluate the success of conservation interventions, providing useful evidence for future efforts. Buxton & Jones (2012) used acoustic monitoring to confirm breeding and document population increases of seabirds after the eradication of introduced Arctic foxes (*Alopex lagopus*) in the Aleutian Archipelago. Similarly, individual identification could be useful in post-release monitoring to determine the fate of specific individuals.

2.4.2 Artificial playback for conspecific attraction

The presence of conspecifics can attract birds to a habitat, but natural conspecific attraction can be unreliable when conspecifics are rare or absent in new habitats (Crates et al. 2017). Artificial playback of vocalizations can be used in place of conspecifics to reinforce existing populations or encourage animals to colonise new areas (Reed & Dobson 1993). This technique has been used to increase local populations of a number of species (e.g., Ward & Schlossberg 2004; Hahn & Silverman 2007), but is not always successful. Bayard & Elphick (2012) found no evidence of a response to broadcast in Saltmarsh Sparrows (*Ammodramus caudacutus*), possibly due to insufficient cues, already saturated habitats, or broadcast in unsuitable areas.

Conspecific attraction could be used to create communities by attracting multiple species simultaneously to a single site (e.g., DeJong et al. 2015). This would be especially useful for colonising newly restored habitats or replenishing protected areas. However, past attempts highlight the need to consider the effects of community change on both target and non-target species. DeJong et al. (2015) found that populations of focal species increased near playback speakers, but populations of some non-focal species declined and suggest that differences in response between species could relate to interspecific competition.

Although artificial playback for conspecific attraction is a simple and cost-effective method, we believe current evidence shows a need for understanding species’ biology and ecology to accurately predict outcomes. Where communities contain multiple endangered species, it is essential to consider the risks associated with conspecific attraction - an increase of one species of conservation concern could lead to the decline of another.

__Table 2.2__: Summary of the uses of bioacoustics in conservation.

Conservation activity	**Use of bioacoustics**	**Possible taxa**	**Potential problem**	**Proposed solution**	**Reference**
Collecting baseline data	detecting species presence or absence, identifying preferred habitats, etc.	species producing vocalizations	lack of standardized methods for acoustic detection surveys	produce standard protocols so studies are comparable	Teixeira et al. 2019
Conducting censuses	individual discrimination to improve resolution	all vocally active species (Terry et al. 2005)	requires all birds to vocalize during census period or for researchers to understand the proportion and demographics vocalizing	research species vocalization prior to census where possible	Reviewed by Terry et al. 2005
Assessing life history traits/fitness	recognition of individual animals	all vocally active species (Terry et al. 2005)	assigning vocalizations to individuals can be challenging	investigate vocal individuality prior to study onset	Reviewed by Terry et al. 2005
Assessing responses to perturbations	detecting presence or absence, changes in activity, differences in biodiversity	species producing vocalizations			e.g., Deichmann et al. 2017
Assessing success of conservation interventions	(as above)	species producing vocalizations			e.g., Buxton & Jones 2012; Metcalf et al. 2019
Attraction of single taxa to new habitats	playback of conspecifics to replicate conspecific attraction	e.g., territorial songbirds, colonial seabirds	unsuccessful attraction of target species	understanding of species' biology and ecology – especially	e.g., Reed & Dobson 1993; Ward & Schlossberg 2004;

			potential declines of nontarget species (particular concern if multiple endangered taxa are present)	habitat, carrying capacity, and community structure	Hahn & Silverman 2007; Bayard & Elphick 2012
Attraction of multiple taxa to new habitats	(as above)	(as above)	(as above)	(as above)	e.g., DeJong et al. 2015

2.4.3 In situ conservation

Large- and small-scale changes in land use can isolate previously contiguous populations, promoting song divergence through drift in the separate populations and through adaptation to changed habitat structure (both for acoustic transmission and morphological features). Closed-ended learners may be particularly vulnerable to habitat change because they would be unable to alter their song to transmit well in the new environment. We suggest that preventing land-use change and protecting species as contiguous populations in the same habitat would help prevent vocal change. Where this is not possible, maintaining or improving connectivity between populations may reduce divergence in song characteristics.

Although limiting the impact of invasive species is a key goal of many conservation efforts, the impact of invasive species on vocalizations is rarely considered. When new species enter the acoustic landscape, resident species may alter song characteristics (Grant & Grant 2010) or become masked by the new vocalisations. Acoustic monitoring of Mediterranean shrubland revealed that the invasive Red-billed Leiothrix (*Leiothrix lutea*) is acoustically dominant in the landscape, competing with and potentially lowering the density of native species (Farina et al. 2013). If native species cannot compete with invaders in the acoustic landscape, they may not be able to adequately communicate and breed. Preventing future invasions is important to maintain acoustic landscapes for conservation of native species.

2.4.4 Translocations

Translocations often involve small founder populations, so the formation of cultural bottlenecks is a concern. Serial translocations, where populations from successful translocations are used as source populations for future translocations, may increase the rate of song differentiation among populations, resulting in population divergence, isolation, and reduced retention of animals near release sites. In the North Island Kokako, in translocated populations songs are shorter and of higher frequency and phrase repertoires are lower than in source populations (Valderrama et al. 2012, 2013). Vocal activity is also markedly reduced, potentially reducing immigration and retention of birds in already small populations (Valderrama et al. 2012). When examining serial translocations in North Island Saddlebacks, Parker et al. (2012) reported reduced song type sharing between translocated and ancestral populations in successive interventions (9.8% shared after the first translocation, 9.2% after the second, and 3.3% after the third). Withdrawal of learning could also cause rapid divergence of songs in recently translocated populations. Moving adult birds with crystallised songs would be preferable to moving juveniles when aiming to limit changes in vocalizations. The withdrawal of learning effect is poorly understood in many species, so monitoring vocalizations after interventions would provide vital information for future conservation programmes.

To improve genetic diversity during interventions, multiple source populations may be used for translocations. However, if source populations have different dialects, this may affect territory formation and mate choice. In the North Island Kokako, which is the subject of intensive conservation management, local songs elicit stronger responses from territory-holding pairs than foreign songs (Bradley et al. 2013), although this result is not consistent among sites (Valderrama et al. 2013). Further examination of responses to dialects across populations is necessary to determine the nuances of differential discrimination. Populations (or individuals) that respond similarly to local and foreign dialects would more easily integrate into new mixed-dialect populations, making them potential targets for conservation interventions. However, choosing birds based on response may result in inadvertent selection for response and associated traits. Similarly, assortative mating with respect to dialect is common in Kokako; across 10 multisource translocations over 18 years (1993-2011) Kokako mated assortatively in most seasons at all five sites. Very few mixed-dialect pairs formed (Bradley et al. 2014), and mixed-dialect pairs took considerably longer to form than matched pairs (Rowe & Bell 2007). The long-term impact of mate selection based on dialect is not clear. First-generation Kokako hatched at translocation sites do not appear to show preferences for their fathers' dialects, lending support to theories on familiarity. However, sample sizes are too small to draw clear conclusions. Two first-generation females paired, one with a male of dialect similar to her father's and the other with a male of a different dialect (Rowe & Bell 2007). Monitoring of mate choice in populations over multiple generations would help determine the overall impact of selection based on dialect over time.

Where dialects are a conservation concern, several mitigation methods could be used: increase starting population size to counteract increased selectiveness; use individuals from the same source population where possible; and select populations with similar dialects to reduce dialect differences in the new group. It is possible that familiarising birds (either adults or juveniles) with different dialects using playback before translocations could alter preferences and reduce selectiveness in mate choice. Macdougall-Shackleton et al. (2001) report that preference for natal-dialect song attenuated in birds exposed to foreign-dialect song when they were one year old. More research is required into the feasibility of this technique. However, if familiarity with dialects reduces aversion to foreign-dialect mates, conservation practitioners may be able to familiarise birds with all dialects in their new population before the interventions take place, thus improving mating success.

2.4.5 Ex situ conservation

Populations in ex situ management are often small and isolated, which may accelerate the rate of cultural drift (Laiolo & Tella 2007). Housing species in larger groups ex situ where possible or retaining greater levels of group connectivity, for example, by moving birds between populations more frequently, could help to combat the effects of drift. Reducing the number of generations birds are held in ex situ populations would also reduce the likelihood of building up large song differences. These mitigations are particularly important for species with complex songs because these may undergo accelerated rates of drift compared to simple songs (Purushotham & Robin 2016).

Breeding birds ex situ may promote song divergence from the wild-type through acoustic adaptation, meaning birds will be poorly adapted when reintroduced. Although captive environments are necessarily different from those in situ*,* matching the environments as closely as possible would limit song evolution. Small changes could be made to increase similarities, such as matching the denseness of vegetation in wild habitats and reducing the presence of anthropogenic noise, such as air-conditioning units. Again, reducing the number of generations birds spend in unfamiliar environments during conservation interventions may also reduce adaptation because acoustic adaptation can increase with time (Potvin & Clegg 2015).

Captive environments may also differ from those in situ due to species compositions. Mixed-species enclosures often hold a range of species that would not overlap in the wild, creating an unnatural acoustic landscape. As with invasive species, birds may alter their vocalizations, but if signals become masked then breeding success in these aviaries may be reduced. Whilst there is currently little research in this area, potential issues could be avoided by housing species in natural assemblages from the same geographic location.

In captive populations, breeding pairs are usually assigned rather than allowed to form naturally to maximise genetic variability. However, animals limited in mate choice often show reduced reproductive success (Martin & Shepherdson 2012). We hypothesise that a lack of acoustic separation from males with high-quality or local dialect songs may alter females’ perceived mate availability, resulting in females reducing reproductive efforts with their assigned mates. Allowing mate choice or providing appropriate acoustic separation could help alleviate this problem and increase breeding success.

Although reducing the number of generations in captivity could act to reduce divergence in vocal communication, this may not always be possible, and some birds have already been conserved ex situ for many generations (e.g., the Alala (*Corvus hawaiiensis*) (Tanimoto et al. 2017)).

Prolonged captive breeding could result in species becoming adapted to captivity. Surprisingly, very few studies examine song differences between wild and captive individuals. Tanimoto et al. (2017) compared vocalisations of the Alala between past wild and current captive populations, finding similar numbers of call types, but significantly different repertoires. Because dialects change over time, it is not possible to say how much of a role captivity played in these changes. All Alala were brought into captivity, so it is not possible to conduct a contemporary comparison.

The song of the domestic Bengalese Finch (*Lonchura striata domestica*) is more syntactically complex than that of its wild counterpart, the White-rumped Munia (*L. striata*) (Honda & Okanoya 1999). Several possible explanations for these differences have been proposed, including lack of predation pressure in captivity for Bengalese Finches (Honda & Okanoya 1999); need for species identification by white-rumped munia in mixed-species flocks (Kagawa et al. 2012); lower levels of corticosterone in Bengalese Finches (Suzuki et al. 2012); and selective breeding for traits correlated with song complexity, such as reproductive output and parental care, in Bengalese finches (Suzuki et al. 2013). Domestication exerts far stronger selection pressures on populations than captive breeding. However, this example highlights some potential mechanisms of change during long-term ex situ management.

Many of the situations that give rise to song divergence, such as release from predation and reduced stress, are unavoidable in captivity. However, the steps outlined above, such as minimising differences in environment and enabling mate choice, may slow or reduce overall change. Breeding management strategies are already commonplace in zoological collections for maintaining genetic and demographic viability (Ballou et al. 2010). These same techniques, such as ensuring individual lineages do not become overrepresented in the breeding pool, may also limit song changes during captive breeding.

2.4.6 Reintroductions

Many issues facing reintroduction efforts stem from breeding ex situ. The negative effects of adaptation to captivity are highlighted by the relative success rates of translocations and reintroductions. Fischer & Lindenmayer (2000) report that 31% of translocations of wild animals have been successful, but only 13% of translocations with captive animals have been successful. Birds with vocalizations adapted to captive environments may signal less efficiently on release to the wild (Tanimoto et al. 2017). We expect this would be more detrimental to closed-ended learners, which would not be able to alter songs for their new environment.

Divergence from wild-type vocalizations may cause problems similar to those seen in translocations with multiple sources if reintroductions aim to supplement preexisting populations, reintroductions use multiple source populations, or multiple reintroductions to a single site are planned. If dialects have diverged, all of these scenarios may affect territory formation or result in increased mate selectiveness and possibly reduced gene flow. Although dialect-based assortative mating between wild and captive birds has not been studied, it may be expected based on previous studies of female preference (e.g., Searcy et al. 2002) and evidence from translocations (Rowe & Bell 2007; Bradley et al. 2014). We speculate that if reintroduced birds are unable to breed with wild populations or to persist as stable populations themselves and if reintroduced birds compete with native populations, then competition created by reintroductions may hasten rather than prevent extinctions.

Improving the success of reintroductions will involve steps in captivity to reduce song divergence, as outlined above (reduce habitat differences, reduce number of generations in captivity). Recommendations for reintroductions are similar to those for translocations: increase the size of release groups, choose birds with similar dialects to each other and to the source population where possible, and use adult birds to prevent rapid change after release. Moreover, exposing birds both in and ex situ to the dialects of other populations may improve integration and mating success, although further research is needed to determine the feasibility of this approach.

Table 2.3: A summary of the implications of avian vocalizations in conservation.

Conservation problem	**Acoustic implications**	**Taxa to consider**	**Proposed solution**	**Reference**
In situ conservation				
Habitat fragmentation	vocal divergence through differential drift and acoustic adaptation in separated populations	vocal learners – parrots, hummingbirds, songbirds, corvids (Catchpole & Slater 2008; Bluff et al. 2010)	conserve species as contiguous populations or maintain or improve connectivity	Laiolo & Tella 2005
Land use and habitat change	vocal change through acoustic adaptation	species producing vocalizations	minimize land-use change where possible or maintain and improve connectivity	Boncoraglio & Saino 2007; Brumm & Naguib 2009
	vocalizations poorly adapted to transmission in the new environment	closed-ended learners e.g., estrildid finches and sparrows (Brenowitz & Beecher 2005)		
Invasive species	competition in the acoustic landscape resulting in signal masking	native species producing vocalizations	prevent future invasions and manage current invasive species;	Grant & Grant 2010; Farina et al. 2013
Translocations				
Small founder populations	formation of cultural bottlenecks, accelerated by serial translocations	populations/species with multiple vocalization types	increase founding population size where possible	Parker et al. 2012; Valderrama et al. 2012, 2013

			select representative sample of vocal diversity when choosing birds to translocate	
Translocation of juveniles	withdrawal of learning resulting in rapid vocal divergence	juvenile birds	use adult birds (or birds with crystallized song) in translocations	Potvin & Clegg 2015
Founding populations from multiple sources	reduced success of territory formation and maintenance assortative mating in translocated populations	birds with vocal dialects (mainly vocal learners, as described above)	increase founding population size to counteract increased mate selectiveness use individuals from the same source where possible select source populations with similar dialects to reduce variation in founding population monitor populations over multiple generations to determine the full extent of assortative mating following the translocation explore the potential of familiarizing birds with other dialects pre-release	Rowe & Bell 2007; Bradley et al. 2013, 2014; Valderrama et al. 2013
Ex situ conservation				
Small, isolated populations	cultural drift may be accelerated in small, isolated populations	vocal learners particular concern for species with complex songs	manage species in larger groups where possible, or retain greater levels of connectivity by moving birds between populations more frequently	Laiolo & Tella 2007; Purushotham & Robin 2016

Differences between wild and ex situ habitats	acoustic adaptation to ex situ habitats different acoustic landscapes due to non-native species assemblages	species producing vocalizations	reduce number of generations birds are kept in captivity increase similarity between in situ and ex situ environments (e.g., denseness of planting, reduce anthropogenic noise, and reproduce native species composition)	Potvin & Clegg 2015
Adaptation to captivity	release from selection pressure resulting in large scale vocal divergence from wild-type	species held in captivity for many generations	reduce number of generations birds are kept in captivity minimize differences between *in situ* and *ex situ* habitats breeding management to ensure that individual ancestors do not become over-represented	Honda & Okanoya 1999; Tanimoto et al. 2017
Reintroductions				
Ex situ breeding	discussed above	discussed above	discussed above	discussed above
Release from ex situ populations	vocalizations poorly adapted to new environments	species producing vocalizations particular concern for closed-ended learners	reduce differences between in situ and ex situ environment reduce number of generations in captivity	Rowe & Bell 2007; Bradley et al. 2013, 2014; Valderrama et al. 2013

	presence of divergent vocal dialects (between wild and released birds or between different populations of released birds)	species with vocal dialects	increase founding population size to counteract increased mate selectiveness select source populations with similar dialects to each other or to in situ population to reduce variation in newly formed populations explore potential of familiarizing birds with other dialects pre-release	

2.5 Conclusion

Considerable progress has been made in the use of vocalizations to aid conservation, particularly with bioacoustics used to monitor populations and survey biodiversity. Whilst research into bioacoustic monitoring continues to grow, research examining the adverse effects of variation in vocalizations during conservation programmes is lacking. There are a number of important questions that should be addressed in order to build a better evidence base for conservation practitioners. First, little is known about the actual effect of dialects on mating. While females often show preference for local songs, this may not reflect how they mate in conservation settings. Although some evidence for assortative mating in the wild exists (e.g., the Kokako), evidence from mixed-dialect populations suggest dialect-based assortative mating may be due only to familiarity. If this is true, it may be possible to mitigate the problem by familiarising young birds with the dialects they may encounter during conservation efforts. Similarly, additional research is needed into the long-term effects of dialect-based assortative mating; assortative mating may not be maintained over multiple generations. Long-term population monitoring after intervention would be necessary to determine this. Furthermore, understanding of the interplay between dialects and gene flow remains limited. Individuals are often closely monitored during conservation to examine breeding success. Thus, it may be possible to determine how dialects affect gene flow by constructing pedigrees within populations. If gene flow persists during interventions in spite of dialects, this knowledge would be extremely beneficial to practitioners. Finally, the evolution of birdsong during ex situ management is poorly understood. Understanding the drivers and extent of acoustic change during conservation breeding is essential for planning of breeding programmes and reintroductions. Future research should focus on these knowledge gaps to help practitioners and scientists properly plan for and mitigate potential adverse effects of variation in vocalizations during conservation.

2.6 Author Contributions

RL wrote the initial draft of the manuscript. All authors contributed to reviewing and editing the manuscript.

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2.8 Discussion

Whilst this review focused on the use of bioacoustics in avian conservation, there is also a range of literature examining these topics in other taxa. This suggests that the applications, problems and potential solutions identified here may have broader applications across taxa. In addition, since the publication of this chapter, new studies on avian taxa have been published that further explore some of the ideas presented in the review. In this section, I discuss how the ideas in Chapter 2 relate to research in other taxa and how they are supported by new research in bird conservation.

In Chapter 2, we discussed the importance of acoustic data in population monitoring and the monitoring of animals during conservation interventions. In a recent review of common difficulties in conservation translocations, difficulties with post release monitoring were cited as an issue faced by managers (Berger-Tal et al. 2020), highlighting the need for simple, effective solutions. Yan et al. (2019) developed an acoustic ethogram using vocalizations and incidental sound to monitor the reintroduction of giant panda (*Ailuropoda melanoleuca*). From recordings taken using microphones mounted on GPS collars, they were able to identify behaviours indicative of breeding success, such as infant suckling. Metcalf et al. (2019) used ecoacoustics to monitor the post-translocation behaviour of the hihi (*Notiomystis cincta*), an endangered passerine. They combined dynamic occupancy modelling with data collected from autonomous recording units. Using this approach, they were able to temporally and spatially model hihi behaviour and identified that the areas hihi chose to settle in were strongly predicted by distance from water. Without acoustic data, this information would have required expensive and more challenging methods to obtain, highlighting the importance of acoustic data for post-release monitoring.

Although not discussed in depth in Chapter 2, these monitoring techniques can also be used during ex situ conservation breeding programmes to monitor animals and to inform animal husbandry and management. Vocal behaviour is conspicuous an may be easily heard and recognised by animal caretakers. As such, it can be used to monitor a range of situations during conservation breeding programmes. Vocal cues can indicate oestrus in a range of species, and has been well studied in domestic animals (e.g., cows (Green et al. 2018)). However, this is also relevant in conservation breeding programmes. In a female slow loris (*Nycticebus* sp.), peaks in vocal activity were associated with oestrus, and these cues were consistent across management conditions (Schneiderová & Vodička 2021). Vocal behaviour may also provide insights into the success of management interventions, such as introducing individuals for breeding purposes. In giant pandas, call usage during introductions varied significantly according to outcome (Charlton et al. 2018). Whilst some call types, such as female moans, were strongly associated with successful outcomes, one call type, the female roar, was indicative of failure and not observed in any successful interactions. Male bleats from successful introductions were longer than in unsuccessful attempts, although this may be harder to identify during general observation and management. Animal caretakers could be trained to recognise vocal patterns associated with success, which may aid them in decisions about continuing introductions (Charlton et al. 2018).

In Chapter 2, we cited differences in the sound environment as a potential driver of vocal change between populations. Such differences have been examined for zebrafish (*Danio rerio*); an important model species frequently kept in captive environments. Whilst this species does not produce vocalizations, underwater soundscapes can play an important role in shaping auditory structures and sensitivity in fish (Lara & Vasconcelos 2019). Soundscapes inhabited by wild zebrafish were varied and variable in terms of soundscape composition, sound pressure levels and spectral features. In general, natural habitats had lower but more variable sound levels than captive environments, which had low variability in sound pressure levels. The distribution of sound energy also differed between the two environments; natural habitats showed most energy concentrated below 600-800Hz, with a peak in noisier habitats at 1000-4000Hz, whereas artificial housing systems had most energy under 1000Hz. The authors suggest that the level and distribution of noise in artificial systems was likely to cause significant auditory masking and had the potential to affect auditory sensitivities. Differences in the soundscape between wild and captive environments have been suggested to contribute to vocal change in golden mantella frogs (*Mantella aurantiaca*) (Passos et al. 2017). The calls of captive frogs differed from those of wild populations across a suite of temporal and frequency-based features (Passos et al. 2017). However, frogs in an in situ breeding centre for a single generation had calls that were less diverged from those of wild frogs. This is consistent with the recommendation in Chapter 2 suggesting that reducing time spent in captivity may help to limit the effects of vocal change. These differences appear to contribute to recognition and response; zoo-bred golden mantella showed a significantly stronger phonotactic response to playback of calls from zoo-bred frogs compared to calls from wild populations. On the contrary, frogs of wild origin showed a similar response to calls regardless of origin. As discussed for birds in Chapter 2, these differences in response could have significant negative implication for conservation efforts if zoo-bred frogs were to be released to the wild (Passos et al. 2017).

An important theme in Chapter 2 was the role of vocal learning in the development and persistence of vocal dialects in endangered species, and how this may influence behaviours that are important in conservation programmes, such as courtship and breeding. Recent research on the regent honeyeater (*Anthochaera phyrgia*) revealed that population fragmentation has resulted in loss of vocal culture in the species. Regent honeyeaters likely learn from adult conspecifics later in life, as male birds do not produce songs while their offspring are resident in their territory (Crates et al. 2021). Therefore, population declines and fragmentation can affect vocal development by reducing interactions with appropriate tutors and in turn opportunities to learn species-specific vocalizations. As songs are involved in mate attraction and territory defence in this species, cultural erosion could reduce population fitness and contribute to the Allee effect. Some individuals exhibited interspecific singing, including vocal patterns from other species in their songs, and the probability of producing these was associated with population density; sparser populations were more likely to sing interspecific songs (Crates et al. 2021). Individuals that were captive bred had simple songs compared to wild-origin birds, again suggesting that conspecific tutors are important for the development of high quality, species-typical songs (Crates et al. 2021). Even within wild origin birds, present day songs were less complex than those previously recorded in the populations, consistent with vocal learning in other declining populations (Valderrama et al. 2012). This may reflect lack of opportunities to learn from conspecifics combined with copying errors by some members of the population, but may also reflect developmental stress from more challenging conditions. Importantly for conservation, song type also influenced paring success; males that sang songs which differed from their regional cultural norm were less likely to be paired with a female, although song type did not affect nesting probability of paired birds or fledging success (Crates et al. 2021). This is similar to patterns of preference for local song dialects seen in other bird species, as outlined in Chapter 2. Unusual vocalizations did not preclude pairing; some interspecific singing males still paired (Crates et al. 2021). Song culture may, therefore be a useful indicator of population health and trajectory, and may be particularly useful for species that are otherwise hard to measure due to its conspicuous nature. These findings suggest that it is necessary to take steps to ensure that reintroductions are successful. As discussed in Chapter 2, exposing birds to conspecific songs prior to reintroduction could improve success of reintroduction programmes. In the regent honeyeater, captive birds exposed to wild origin song had higher survival when reintroduced (Tripovich et al. 2021). Despite this, the effect on breeding success is less apparent. Whilst no variables measured affected female breeding success, males that were exposed to conspecific song had a lower chance of breeding success. However, this pattern was no longer apparent when only those males that were resighted during the breeding season were included (Tripovich et al. 2021). Since individual’s vocalizations can show some change over time, tracking over long periods may help to determine if there is behavioural modification post release that could affect survival and breeding. Interestingly, although in Chapter 2 the potential for reducing the number of generations spent in captive environments may improve outcomes during intervention, regent honeyeaters with less generational time in captivity did not have improved outcomes (Tripovich et al. 2021). It is possible that effects may become apparent if more time passes, but the findings of this study suggest that short periods in captive conditions may not adversely affect individuals when released.

In Chapter 2, we discussed the importance of birds being able to correctly respond to vocal cues in a range of situations, such as territory defence and mate choice. As mentioned, the Hawaiian crow (alala; *Corvus hawaiiensis*) exhibited changes in vocal repertoire following ex situ conservation efforts (Tanimoto et al. 2017). This included the loss of territorial broadcast calls, which were common in the wild repertoire, but absent in the captive repertoire. Past wild birds also had around twice the number of alarm calls compared to captive birds. Therefore, it is important to determine if Hawaiian crows in conservation breeding programmes retained their natural responses to these socially relevant calls (Sabol et al. 2022). This would be especially important if birds were to participate in reintroduction programmes in the future. Sabol et al. (2022) examined the response of Hawaiian crows in conservation breeding programmes to alarm and territorial inclusion calls using playback trials. Birds were still able to distinguish between natural call types and respond appropriately. Birds were more likely to approach in response to alarm calls, suggesting threat investigation. In addition, birds responded to territorial intrusion calls with their own territorial intrusion calls, a behaviour more common in males than females, suggesting they were willing to defend their territories (Sabol et al. 2022). However, there was some evidence that individuals differed in their levels of response, with many of the birds not responding to playbacks. It was suggested that due to housing with high social density may result in some birds becoming desensitized to conspecific calls (Sabol et al. 2022). In this case, it would be beneficial to include whether an individual appropriately responds to conspecific calls when determining fitness for release.

Overall, evidence from birds and other taxa suggest that vocal behaviour has an important role to play in conservation programmes across a broad spectrum of taxa. Studies to date suggest that there may be simple ways to avoid issues, such as careful selection of individuals and pre-release training. However, different taxa and species are likely to have specific challenges depending on their life histories and reliance on acoustic communication. As such, further studies are needed to investigate the full impact of vocal behaviour and responses to vocal behaviour on conservation efforts.

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Chapter 3: Like father like son: Cultural and genetic contributions to song inheritance in an estrildid finch

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3.1 Chapter Summary

An important first step in determining the extent to which bird song could change during ex situ breeding programmes is to understand how song is inherited. Vocal learning is a key component of song development in oscine passerines (Beecher & Brenowitz 2005), with untutored birds producing atypical vocalizations (Price 1979; Marler & Sherman 1985; Chaiken et al. 1993; Feher et al. 2009; Kagawa et al. 2014). As discussed in Chapter 2, vocal learning can contribute to the build-up of differences between populations, and therefore influence conservation programmes. However, song development can also be affected by other factors, which could contribute to differences in vocal behaviour between populations.

Genetic background can influence song production and development both directly (e.g., syllable production in canaries (Wright et al. 2004; Mundinger 2010; Mundinger & Lahti 2014)) and indirectly (e.g., through morphology (e.g., Podos 2001)). Genetic change during captive breeding is well reported across a broad range of taxa, and reducing adaptation to captivity and maintaining genetic diversity are often stated as key goals during conservation breeding programmes (Frankham 2008). However, differences between populations may also build up due to stochastic processes, such as drift. As such, if genetic background influences song development, it could contribute to differences between populations.

Additionally, the developmental environment can influence song development. Developmental stress, such as poor nutrition and sibling competition can contribute to reduced song performance, which persists into adulthood (Nowicki et al. 2002a; MacDougall-Shackleton & Spencer 2012). We might expect the developmental environment to differ significantly between populations in and ex situ due to a range of factors, such as food quality and availability, so this could contribute to differences between wild and captive populations.

In this chapter, I examine song inheritance in laboratory population of Java sparrows (*Lonchura oryzivora*), using archival data from multiple generations. Within the dataset, some birds were raised by their genetic father and some were cross-fostered, making this a useful dataset to examine the contributions of different inheritance pathways. I segmented songs and classified notes to allow for comparison across multiple song types within the population and examined a range of song features relating to song structure, acoustic parameters, and consistency to determine the relative contributions of social learning, genetic inheritance, and the developmental environment. Although the correlation of some song features between fathers and sons has been examined previously (Ota & Soma 2014), this study represents the first comprehensive examination of a range of song inheritance mechanisms across multiple facets of the song.

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The work presented in this chapter represents the author accepted version of the manuscript formatted for inclusion in the thesis, with edits for clarity as requested by the examiners.

3.2 Abstract

Social learning of vocalizations is integral to song inheritance in oscine passerines. However, other factors, such as genetic inheritance and the developmental environment, can also influence song phenotype. The relative contributions of these factors can have a strong influence on song evolution and may affect important evolutionary processes, such as speciation. However, relative contributions are well described only for a few species and are likely to vary with taxonomy. Using archived song data, we examined patterns of song inheritance in a domestic population of Java sparrows (*Lonchura oryzivora*), some of which had been cross-fostered. 676 songs from 73 birds were segmented and classified into notes and note subtypes (N = 22,972), for which a range of acoustic features were measured. Overall, we found strong evidence for cultural inheritance of song structure and of the acoustic characteristics of notes; sons' song syntax and note composition was similar to that of their social fathers and was not influenced by genetic relatedness. For vocal consistency of note subtypes, a measure of vocal performance, there was no apparent evidence of social or genetic inheritance, but both age and developmental environment influenced consistency. These findings suggest that high learning fidelity of song material, i.e., song structure and note characteristics, could allow novel variants to be preserved and accumulate over generations, with implications for evolution and conservation. However, differences in vocal performance do not show strong links to cultural inheritance, instead potentially serving as condition dependent signals.

3.3 Introduction

Social learning is an essential component of normal song development for oscine passerines (Beecher & Brenowitz 2005). In many species, birds that are not exposed to tutor song during the sensitive phases of song ontogeny develop atypical vocalizations, exhibiting unusual note structures, decreased stereotypies, abnormal song length and other temporal abnormalities (Price 1979; Marler & Sherman 1985; Chaiken et al. 1993; Feher et al. 2009; Kagawa et al. 2014). Similarly, birds that are tutored by heterospecifics may incorporate song features of the tutor species in their vocalization, rather than solely producing species-typical songs (Johannessen et al. 2006; Eriksen et al. 2009; Mann et al. 2020). In this way, cultural inheritance of vocal behaviour can lead to vocalizations that are shaped by a bird's social environment (e.g., Greig et al. 2013).

However, a number of other factors also influence song development in juvenile birds. Genetic factors can guide song learning and development. For example, in canaries (*Serinus canaria*), genetic differences show complex interactions with learning and song production, influencing the proportion of low- and high-pitched syllables (Wright et al. 2004; Mundinger 2010; Mundinger & Lahti 2014). Genetic factors can also interact with the environment. In Bengalese finches, juveniles produced more accurate imitations of an experimental tutor's song if songs were played back at a tempo that was similar to their genetic father's song (Mets & Brainard 2018, 2019), and the influence of genetic background and environment differed between passively and socially tutored birds (Mets & Brainard 2018). Other heritable traits, e.g., morphology and neural anatomy, can influence song production. Morphological characteristics, such as body size (Kirschel et al. 2009; Kagawa & Soma 2013; Derryberry et al. 2018; García & Tubaro 2018), beak morphology (Podos et al. 2004; Kirschel et al. 2009; Derryberry et al. 2018; García & Tubaro 2018) and syrinx morphology (Elemans et al. 2015; Christensen et al. 2017) are often correlated with song characteristics. The developmental environment, mediated by social interactions and resource availability, also plays a key role in song development. As song production may incur neural costs during development (Gil & Gahr 2002), early developmental stress, such as sibling competition (Soma et al. 2006) or nutritional stress (Nowicki et al. 1998), may influence adult songs. Social interactions may guide song production through fraternal inhibition (Tchernichovski & Nottebohm 1998) and social reinforcement from parents (Carouso-Peck et al. 2020). Finally, both laying order and maternal androgens may contribute to song development (Soma et al. 2009).

Bird song is thought to advertise the relative quality of the singer and to that effect plays an important role in sexual selection (Gil & Gahr 2002). Songs are multi-faceted signals with learned and unlearned features. Consequently, different aspects of song can reveal different information about the singer's quality. The aspects of quality these traits convey depend partly on their pattern of inheritance. Learned or environmentally influenced traits may reveal information about developmental environment or learning ability (Nowicki et al. 2002b, 2002c; Boogert et al. 2008; Zann & Cash 2008), whereas genetically inherited traits may signal 'good' genes, which will be inherited regardless of tutor (Hasselquist et al. 1996; Forstmeier et al. 2009). Both types of traits may also inform potential mates of direct benefits, such as adaption to the local environment (Podos & Warren 2007; Snowberg & Benkman 2007; Badyaev et al. 2008; Branch & Pravosudov 2015) or ability to provision offspring (Buchanan & Catchpole 2000; Halupka & Borowiec 2006; Bartsch et al. 2015). Of the various song features, significant attention has been paid to three categories: song structure, acoustic characteristics of notes, and song performance measures, which demonstrate complex inheritance patterns and provide a wide range of information about singer's quality (Table 3.1).

Table 3.1: Inheritance patterns of common song features

Song feature	Measurement	Role of social learning	Role of genetic inheritance	Role of developmental environment
Complexity	Repertoire size (number of note types, song types etc.) (Searcy 1992) Syntactical complexity (note-to-note transitions) (Honda & Okanoya 1999)	Generational overlap in repertoire and note sequences in normal and cross fostered individuals suggest a learned component (Grant & Grant 1996; Soma 2011)	Genetic predisposition for learning certain song components (Wright et al. 2004; Mundinger & Lahti 2014)	Song learning may incur costs during development, and developmental stress early in life may influence song characteristics and learning (Gil & Gahr 2002; MacDougall-Shackleton & Spencer 2012; Schmidt et al. 2014)
Spectral and temporal characteristics	Acoustic characteristics of notes (frequency, duration, amplitude, etc.) (Catchpole & Slater 2008)	Learning of notes may result in replication of acoustic features of tutor (Ritschard & Brumm 2011). Learned components may reflect local adaptation (Podos & Warren 2007)	Inherited components may reveal singer quality (e.g., body size, genetics) (Forstmeier et al. 2009; Hall et al. 2013)	Stress in early development may influence note production and reduce note copy accuracy (MacDougall-Shackleton & Spencer 2012)
Performance	Song rate, song amplitude, duration, trill performance (Cardoso 2017; Podos & Sung 2020) Song and/or note consistency (Botero & de Kort 2011a; Sakata & Vehrencamp 2012)	Complex interaction between tutor learning and individual quality; low quality birds may not be able to reproduce high performance models (Botero & de Kort 2011a), high quality pupils may increase performance of low quality models (Lahti et al. 2011)	Song performance may correlate with heritable features e.g., body size (Ballentine 2009; Kagawa & Soma 2013) Genes and gene x environment interactions could affect feedback processing and other factors influencing performance (Sakata & Vehrencamp 2012)	Song production involves coordination of complex motor patterns, high energy requirements and physical constraints and may be more indicative of current condition (Gil & Gahr 2002; Botero & de Kort 2011b; Schmidt et al. 2014)

Although song inheritance patterns are well understood for a handful of model species, whether these patterns replicate more broadly across species, and particularly in rapidly diverging lineages and species of conservation concern, is not known. We studied patterns of song inheritance in the Java sparrow (*Lonchura oryzivora*), an estrildid that is endangered in its native range but common in captivity and invasive in some locations (BirdLife International 2018). We examined an existing song library (Kagawa & Soma 2013; Ota & Soma 2014; unpublished data) with songs from multiple generations of father-son pairs for which the genetic pedigree was known. Some birds in our data set were reared by their genetic fathers, and others were cross-fostered by social fathers with songs that were also included in the data set. We mined our data for patterns of social learning, genetic heritability, and environmental effects on the development of song structure, note spectral and temporal characteristics, and vocal performance measures, and we quantified evidence for the patterns that we found. Based on previous findings in this and other species, we expect that 1) song structure will be socially inherited, 2) spectral and temporal characteristics of notes will be socially learned but may also have some non-learned components and 3) vocal performance, measured as vocal consistency, will be genetically inherited.

3.4 Methods

3.4.1 Study population and recordings

The Java sparrow is an estrildid finch native to South East Asia, and is commonly kept in captivity (Restall 1996). Males learn to sing a single song type, typically containing 2-8 note types, during a critical period in the nest, with song learning estimated to end at around 150-180 days (Ota & Soma 2014). As in other estrildids, songs are only used as part of courtship displays (Kagawa & Soma 2013), which in Java sparrows also include duet dancing (Soma & Iwama 2017). Song learning requires social interactions and, as such, juveniles in laboratory settings are most likely to learn from their social fathers if they do not interact with other adult males (Soma 2011). Inspection of spectrograms suggests that sons produce copies of their fathers' songs (Soma 2011), but the relative contributions of cultural and genetic inheritance, and the rearing environment have not been assessed.

We examined song inheritance in a laboratory population of Java sparrows (Hokkaido Univ.) with a known genetic pedigree (Figure 2.1A) and known social relationships between males (Figure 2.1B). Founding individuals were obtained from a range of pet shops and breeders. When breeding, each pair was kept in a separate breeding cage. Nests were inspected regularly, and eggs were cross-fostered when multiple nests with eggs were available. During rearing, each cage was visually, but not audibly, isolated and juveniles remained in the cage with their social parents until they were approximately 180 days old. This ensures that song learning is from the social father only (Soma 2011). See Appendix 1 for further details on subjects and housing.

Recordings were selected from archival data collected between 2011 and 2020. All recordings were made with 44.1 kHz sampling rate and 16-bit resolution and saved as WAV files. Recordings consist of individual birds singing alone in a soundproof chamber. Recordings were taken using digital sound recorders with built-in microphones, which were placed ~20 cm from the bird's cage. Several different recorders were used for the archival data collection (Marantz PMD 661, Zoom Q3HD, TASCAM DR-100 MKIII).

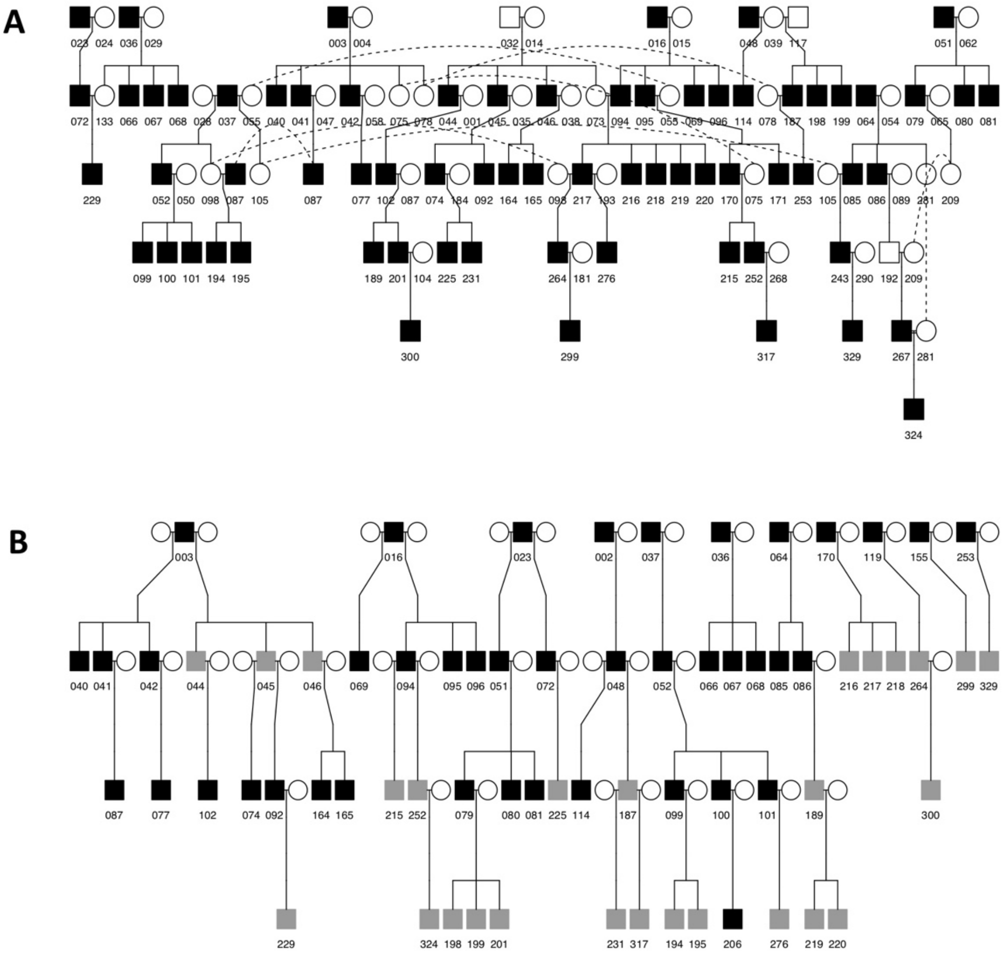


Figure 3.1. Genetic (A) and social (B) pedigrees of Java sparrows included in this study. Squares indicate males and circles indicate females. Numbers indicate bird identity. Filled (open) squares indicate that songs for that male were (were not) available for study. Grey squares in the social pedigree indicate individuals that were cross-fostered. Dotted lines in (A) connect the same individual where it appears multiple times in the pedigree. In the social pedigree (B), the identities of social mothers are not known. Separate clutches in (B) are represented as having different social mothers in the pedigree.

3.4.2 Song selection

Our data set included 58 father-son pairs for which the songs of both the son and the social father had been recorded. Of these, 28 sons were raised by their genetic fathers and 30 were raised by social fathers that were not their genetic fathers (see Figure 3.2 for example songs). Archive data also included a small number of birds that had the opportunity to learn from multiple tutors. These birds were not included as 'sons' in the dataset, as we could not ensure their song learning was confined to the social father. However, birds with multiple tutors exhibited normal adult song and were included in the dataset as fathers if they raised or fathered sons. Altogether, the data set included 73 birds for which songs had been recorded: the 58 sons identified above and 15 additional birds that had raised (social father) or fathered (genetic father) sons but for which the father's song was not available. Thirty-one birds entered the dataset as both fathers and sons.

For each bird, we studied songs recorded within a single week. This is important because song features change with age in some species, including other estrildid finches (e.g. Kao & Brainard 2006; Ballentine 2009; de Kort et al. 2009; James & Sakata 2014, 2015, 2019). If a bird was recorded at multiple time points, then recordings from 'middle' age (~2-5 years) were preferentially chosen. If multiple recording dates were available within this time frame, then one time point was chosen at random. If at least 8 suitable songs were available from the chosen time point, songs from this time point were used in analyses. If a time point with 8 or more songs could not be found when a bird was 2-5 years old, then we first chose recordings where the bird was older than 2-5 years, and only chose recordings from 1-2 years when older recordings were not available. Recordings in which the bird was over one year old were preferred, even if more full songs were available when the bird was younger, since some changes in singing behaviour are apparent between song crystallization and one year of age (Ota & Soma 2014). Across all birds, where more than 10 full songs were available from a single time point, 10 songs were randomly selected. If fewer than 10 songs were identified in every time point for a particular bird, all songs from one time point were used. Overall, this resulted in a total of 676 songs from 73 individuals (average of 9.3 songs per individual, range 3-10, only 3 individuals with <5 songs). The age of birds at recording ranged from 0.41 to 8.83 years, with an average of 3.2 years, and with seven birds recorded at <1 year of age (sons: mean age = 3.1 yrs, range=0.41 – 8.85 yrs; fathers mean age = 2.58 yrs, range = 0.41-6.02 yrs).

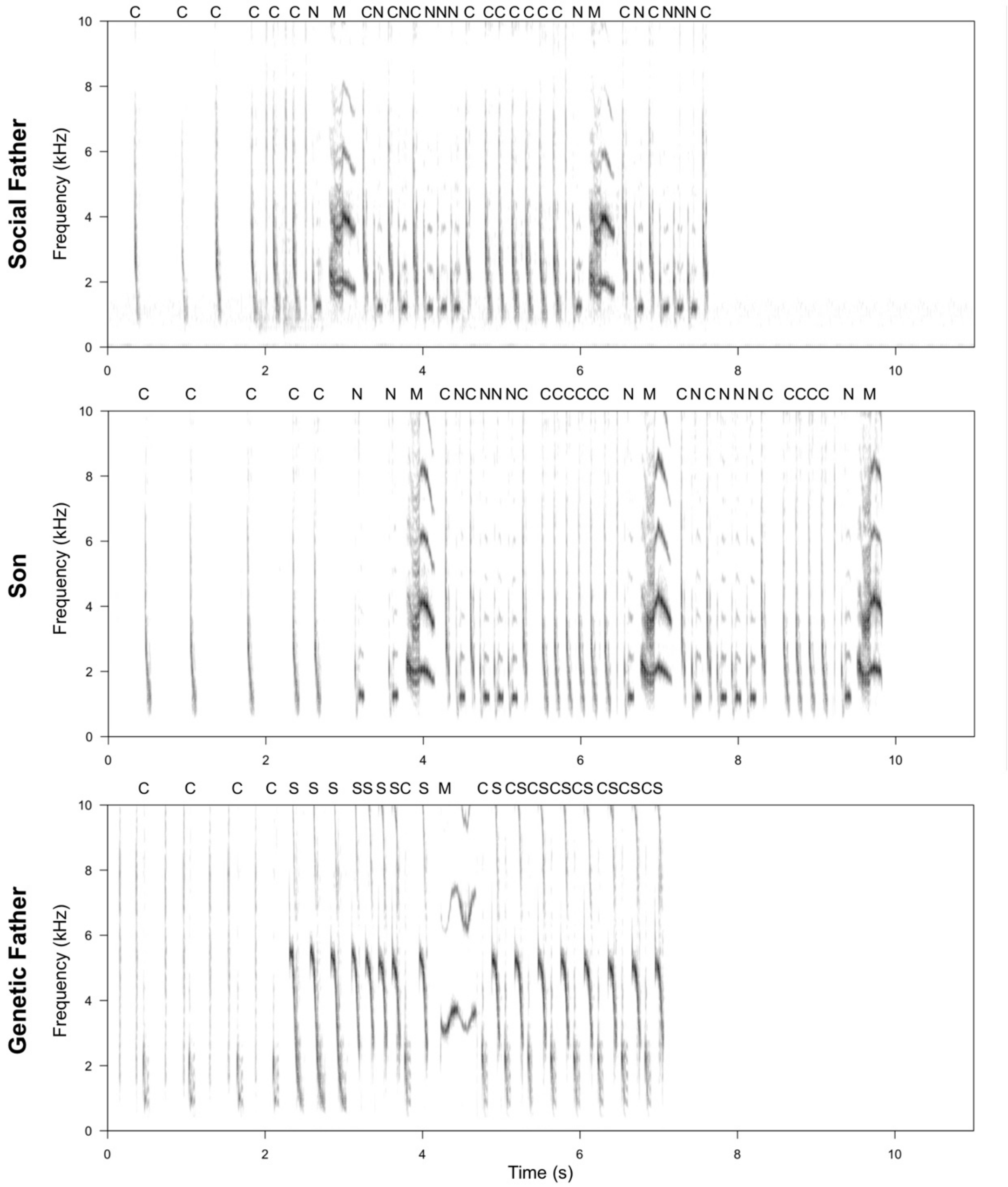


Figure 3.2. Example of a spectrogram comparison of a son's song compared to that of his social and genetic father. Letters above the spectrogram represent note types. Spectrograms were produced using SEEWAVE package (Sueur et al. 2008) (window length=512, overlap=50%). The son produces 100% of the note types in the social father's song (C, N, M), including one that is not sung by the genetic father (N). However, one note type produced by the genetic father is not included in the son's song (S). Transitions between note types in the son's song are more similar to those in the social, rather than genetic, father's song, with 100% of social father's transitions represented, compared to only 17% of genetic father's transitions. Where note types are present in all three individuals, visual inspection suggests that the acoustic characteristics of notes produced by the son more closely resemble those of the social father (particularly apparent for note type M)

3.4.3 Note classification and measurement of acoustic characteristics

3.4.3.1 Segmenting songs into notes

Songs were manually segmented into individual notes using the sound analysis software Koe (Fukuzawa et al. 2020) (window length = 512, window overlap = 50%, time-axis zoom = 400%, contrast 100%). Mechanical sounds like bill clicks (Soma & Mori 2015) were excluded from our analyses and we focused on the production and inheritance of vocal sounds (i.e., notes). A song was defined as a series of notes with inter-note-intervals (gap between notes) of <1 s (Kagawa & Soma 2013). Manual note selection can introduce measurement error (Zollinger et al. 2012), particularly if recording methods are not consistent. However, the recordings used in this study were taken in controlled conditions with minimal background noise, so the beginnings and ends of individual notes could be easily identified.

3.4.3.2 Manual note classification

Notes were classified based on a suite of characteristics (e.g., presence of harmonics, frequency modulation, length and presence of non-linear phenomena), resulting in 15 note types (Figure 3). In total 22,972 notes were segmented and classified (as in Figure 3.3). A second observer who was naïve to Java sparrow song reclassified a random subset of songs (2 songs per individual, 146 songs total, 4915 notes) to determine the repeatability of manually assigned note types. The second observer was provided with a definition of each note type and 8 example notes of each type shown at 100% and 400% time axis zoom. Inter-observer repeatability was high, with agreement of 97.5%.

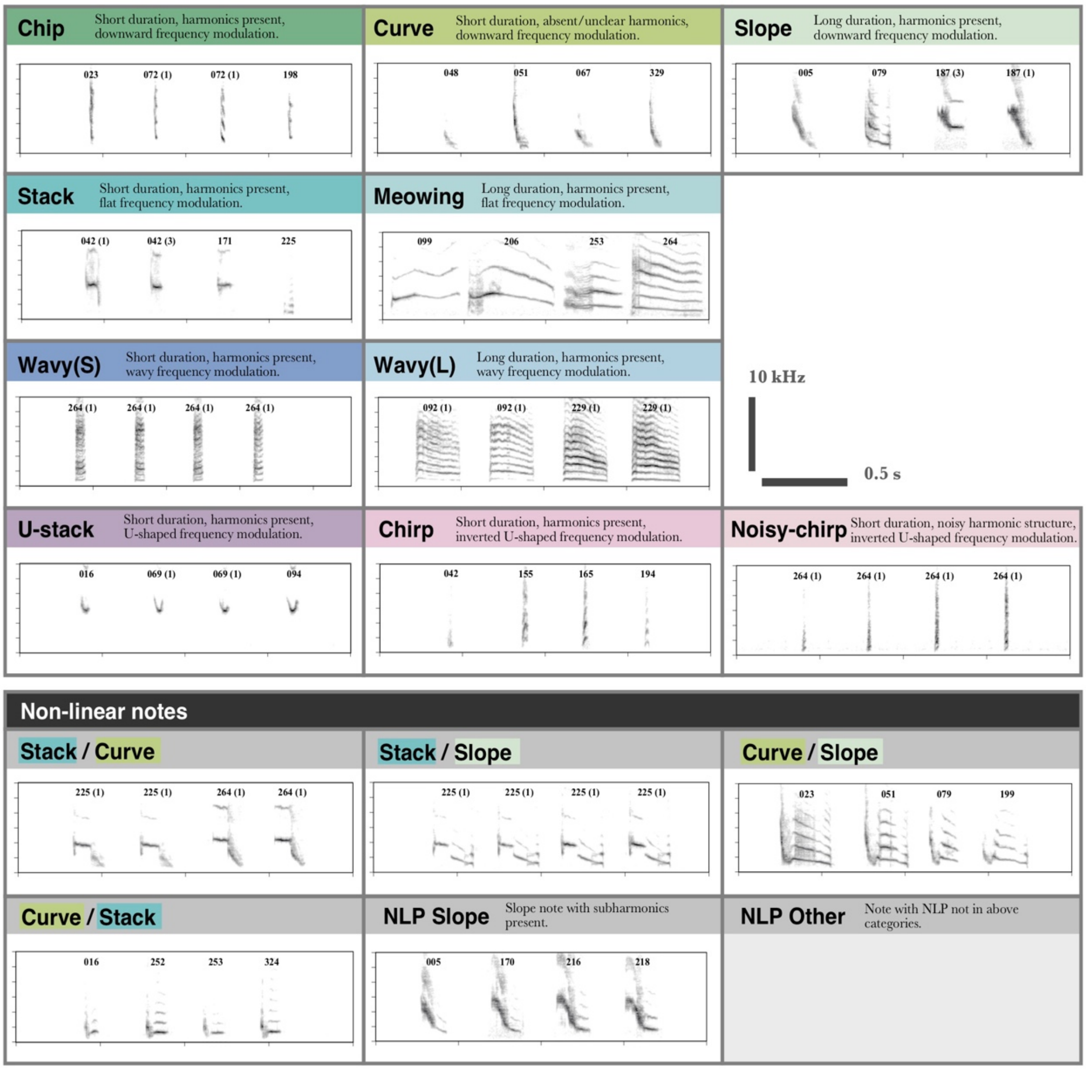


Figure 3.3. Categories used for note type classification. Note type categories were defined based on frequency modulation, harmonic structure, length and presence of non-linear phenomena. Notes are labelled to indicate the individual that produced them. Notes produced by different individuals are automatically classed as different subtypes, as subtypes were not aligned between individuals. Where multiple examples from a single individual are shown the subtype is indicated in brackets.

3.4.3.3 Computational note classification

The notes belonging to a manually assigned note type and produced by an individual bird may not be monomorphic. Rather, note types may be partitionable into subtypes with distinct acoustic characteristics (Figure 4). These subtypes are broadly comparable with 'syllables' in studies of other bird species (Catchpole & Slater 2008). It is not clear whether note types or subtypes are more biologically relevant, so we studied both in our analyses. We classified notes to subtypes by Gaussian mixture modelling using the R package mclust (Scrucca et al. 2016). We based our classification on a subset of three characteristics – duration, mean dominant frequency, and dominant frequency change. We chose these characteristics because they can be reliably measured even for very short notes (i.e., <70 ms), and we needed to classify all notes in the data set to analyse song structure. For each set of notes belonging to a particular note type and produced by an individual bird, we fit Gaussian mixture models with up to 9 clusters, and chose the optimal number of clusters to minimise the Bayesian Information Criterion of the fitted model. We assigned each note to the cluster to which it was most likely to belong, and we called these clusters "subtypes." If a bird produced a note type fewer than five times, we assumed that all notes of that type produced by that bird belonged to a single subtype. We did not attempt to equate subtypes produced by different birds. Subtypes produced by different birds may be overlapping, partly overlapping, or may not overlap at all (Figure 3.4), and therefore equating subtypes produced by different birds is not straightforward. Therefore, direct comparisons between note subtypes produced by different birds, e.g., to assess the cultural inheritance of acoustic characteristics at the level of note subtypes, were not possible.

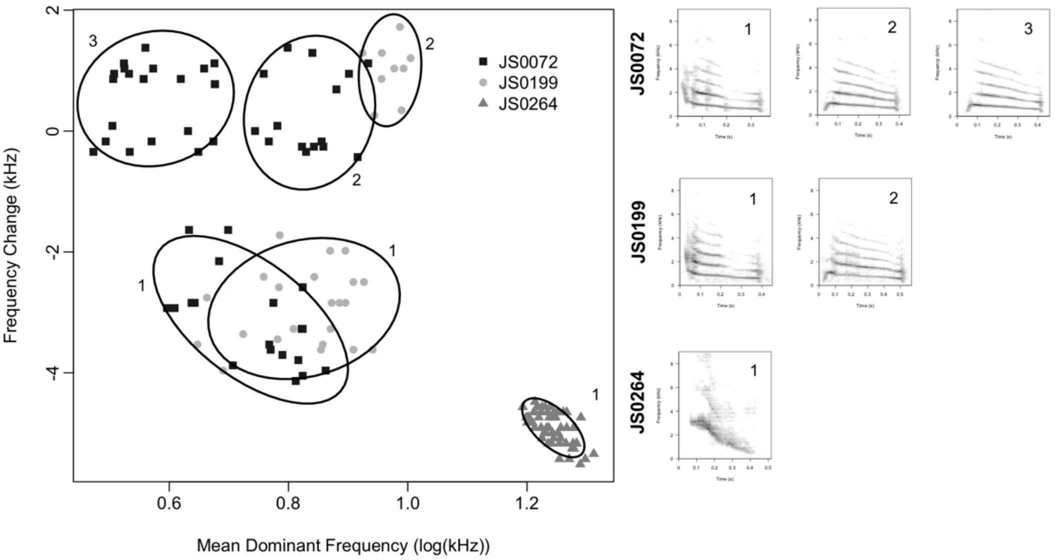
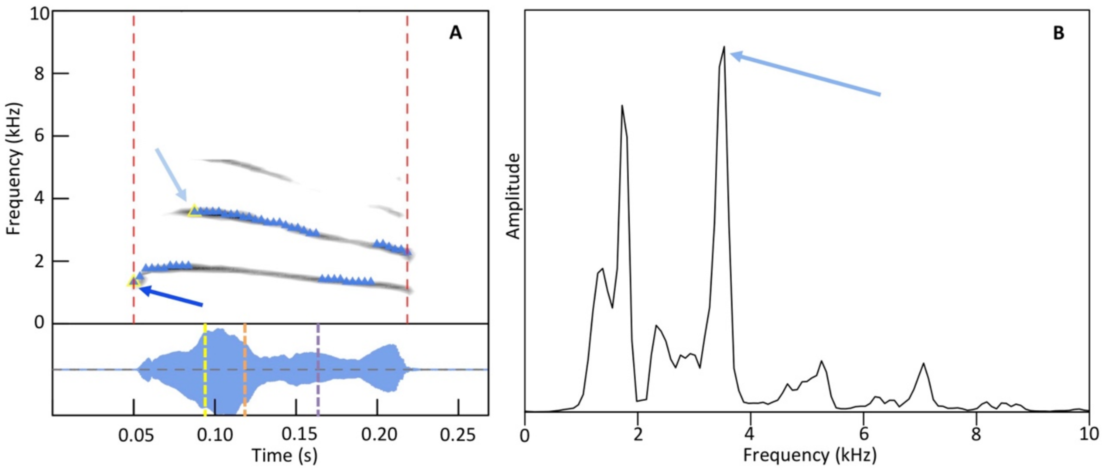


Figure 3.4. Subtypes observed within a single note type for three representative Java sparrow males from this study. Ellipses show the 80% inclusion space for each cluster. Subtypes are labelled within birds and example spectrograms of each subtype for each bird are included. Subtypes produced by different birds may be distinct or partly overlapping. Thus, it is not clear whether clusters represent different notes, or the same note sung differently. For ease of representation, we show only two note features (mean dominant frequency and frequency change), but patterns are similar for other combinations of features.

3.4.3.4 Measurement of spectral and temporal characteristics

To measure the acoustic (i.e., spectral and temporal) characteristics of notes, recordings were first high-pass filtered using a FIR filter at 375Hz to remove low frequency background noise. For each note, we used the specan function (frequency range 0.4 - 22.05 kHz, window length = 512, overlap = 50%, amplitude threshold = 2%) in the warbleR package (Araya-Salas & Smith-Vidaurre 2017) in R (version 3.6.3, (R Core Team 2022)) to measure acoustic characteristics. Specifically, we measured i) the mean dominant frequency of the selection, ii) the dominant frequency change, iii) the maximum dominant frequency in the selection, iv) the modulation index, v) the peak frequency within the selection (based on the mean frequency spectrum), vi) the note duration, vii) the time median, and viii) the time interquartile range (IQR) (Figure 3.5) (see Araya-Salas & Smith-Vidaurre (2017) for further information). We log transformed the note duration and the spectral characteristics (*i.e.*, mean dominant frequency, maximum dominant frequency, and peak frequency) to homogenise variance. We double log transformed the modulation index, and then set values with no modulation to the smallest detectable modulation in the data set (i.e., Winsorizing; Tukey 1962). Double log transformation makes units difficult to interpret. However, because our goal is to regress the acoustic characteristics of sons' notes on the same acoustic characteristics in the notes of their social fathers, the regression coefficients in our analyses are unitless. We normalized the time median and the time IQR by dividing them by the duration of the notes in which they were measured to obtain values between zero and one. This ensures that the measurement of the energy distribution over time is independent of the note duration.



Measure	Description
Duration	Time between red dotted lines in A (s)
Mean dominant frequency	Mean of dominant frequency values (blue triangles) measured across the note (kHz)
Maximum dominant frequency	Highest dominant frequency in the note (pale blue arrow) in A (kHz)
Dominant frequency change	Starting dominant frequency (1st blue triangle) – end dominant frequency (last blue triangle) in A (kHz)
Modulation index	Cumulative absolute difference between adjacent dominant frequency values (adjacent blue triangles) divided by dominant frequency range (difference between points indicated by blue arrows) in A
Peak frequency	Frequency of highest energy within the selection (blue arrow in B)
Time median	Time at which note is split into two parts each containing 50% of the total energy of the signal in A (orange dotted line) (s)
Time IQR	Third quartile time (time at which signal is divided into two intervals of 75% and 25% energy respectively) minus first quartile time (signal divided in two time intervals of 25% and 75% energy respectively) (purple dotted line – yellow dotted line) in A (s)

Figure 3.5. Definitions of acoustic characteristics measured for each note

3.4.4 Data analysis

3.4.4.1 Song structure analysis

We studied the inheritance of song structure computed at the levels of i) note types and ii) note subtypes. We represented each song as a series of note (sub)types, and computed the number of notes, the number of note (sub)types (i.e., repertoire size), the Shannon index, the sequence linearity (Scharff & Nottebohm 1991), and the first and second-order differential entropies (Schmitt & Herzel 1997) at each level. One note type was not reliably classified into subtypes by our Gaussian mixture models, and we assigned all instances of this note type to a single subtype for analyses. For each bird, we took the mean of each song structure measure across all songs in the dataset to obtain a single phenotype per bird and per measure. We regressed the sons' phenotypes on the phenotypes of the social fathers. A significant positive regression coefficient indicates that the phenotype is culturally inherited. In particular, coefficients close to one indicate little regression to the population mean, so the characteristics of a song lineage are likely to persist for many generations, and regression coefficients close to zero indicate that the characteristics of a song lineage rapidly decay towards the population mean. To test for genetic heritability, we included a random effect of relatedness in the regression, where the relatedness matrix was computed from the known pedigree of birds in the data set. Including the full relatedness matrix rather than just the genetic father in our analysis allows us to take advantage of information about more distantly related individuals, and increases our ability to detect genetic effects. This is particularly important because some birds were raised socially by their genetic fathers, which makes it difficult to disentangle social learning from genetic inheritance without considering similarities among more distant relatives. A significant effect of relatedness would indicate that, even when controlling for potential learning from the social father, birds' song phenotypes were more similar to those of related than those of unrelated individuals. We included a random effect of the clutch ID to account for similarities among nestmates that are not due to learning from the social father. This could be due to factors including, but not limited to, common rearing conditions, differences in the quality of parental care, the sizes of broods, and the identity of social mothers (whose song preference and social feedback may influence the song learning and production of her sons (Carouso-Peck & Goldstein 2019; Carouso-Peck et al. 2020)). Because Java sparrow songs can change with age following crystallization (Ota & Soma 2014), we included a fixed effect of log-transformed age in the model. We removed the effect of age from the model if it not at least marginally significant (i.e., $p>0.1$). In this and subsequent analyses, we log-transformed the song phenotypes and refit the models if necessary to homogenise variance in the residuals. We fit models using the lmekin function in the R package coxme (Therneau 2018), and we tested the significance of random effects using likelihood ratio tests. Likelihood ratio tests of random effects are known to be conservative (Pinheiro & Bates 2000).

3.4.4.2 Analysis of acoustic characteristics

Next, we asked whether the acoustic characteristics of sons' notes were similar to those of their social fathers when they produced the same note types. We computed the mean value for each characteristic of each note type as produced by each bird. If a bird did not produce a particular note type at least five times, then we excluded that note type from the analysis for that bird. Thus, if an individual bird produced four different note types at least five times each, then we computed four means for that bird. The variances of acoustic characteristic values for the note types in our data set differed by up to three orders of magnitude. We z-scored acoustic characteristic values within note types to homogenize variance, as homogeneity of variance is a fundamental assumption of our regression models. Finally, for each acoustic feature, we regressed the sons' mean for each note type on the social fathers' mean for the same note type. If a social father produced notes of a particular type but his son did not, or *vice versa*, then that note type did not appear in the analysis for that social father-son pair. A significant positive relationship between the social fathers' mean acoustic characteristic value and the sons' mean acoustic characteristic value indicates that sons learned how to produce individual note types from their social fathers. We included fixed effects of note type and log-transformed age in the model, and we included random effects of the relatedness matrix, the clutch ID, and the individual ID of the son. Including a fixed effect of note type accounts for the fact that different note types have different mean characteristic values, and prevents us from inferring that fathers and sons produce notes with similar characteristics simply because they produce the same note types. The effect of age accounts for the possibility that older birds produce notes differently than younger birds. We removed this effect from the model if it was not at least marginally significant (i.e., $p>0.1$). The random effect of the relatedness matrix controls for potential heritability of acoustic characteristic values, and the effects of clutch and individual control for correlations among sons' acoustic characteristic values that are not due to learning from their social fathers.

3.4.4.3 Performance analysis

The ability to produce individual note types consistently is thought to be a signal of mate quality in a number of species, and birds are likely to compare notes that are produced within the same song (Botero & de Kort 2011a; Sakata & Vehrencamp 2012). Therefore, we wanted to know whether the ability to produce note types consistently within a song is culturally transmitted, genetically heritable, and/or influenced by the rearing environment. We cannot study consistency at the level of note types. Some birds produce multiple note subtypes within note types. If sons learn which subtypes to produce from their social fathers, as our results suggest they do, then studying consistency at the level of note types will confound the learning of note type consistency with the learning of note subtype. Therefore, we studied consistency at the level of note subtypes. To achieve this, we i) assessed the within-song consistency of each note subtype produced by each bird, ii) standardised across note subtypes to control for the fact that some note subtypes may be more difficult to produce consistently than others, iii) computed the mean consistency for each bird across all note subtypes that the bird produced, and iv) regressed the consistency of sons on the consistency of their social fathers.

We assessed the consistency of note subtypes in three ways: by comparing i) the variance of individual acoustic characteristics, ii) the dynamic time warping distance, and iii) the spectral cross correlation among notes. For the variance measures and dynamic time warping distance, lower values indicate greater consistency, and for spectral cross correlation, higher values indicate greater consistency. For each song produced by each bird, we calculated the variance of the acoustic characteristics of each note subtype that appeared in that song more than once. We examined the same acoustic characteristics that we used previously to classify notes to subtypes (i.e., the logarithms of duration, mean dominant frequency, and change in dominant frequency over the course of the note). Within each song produced by each bird, we measured the mean squared pairwise dynamic time warping distance and the median pairwise cross correlation between notes of the same subtype. At the assessed window length (512), warbleR does not accurately measure the change in dominant frequency for notes less than 20 ms in duration, so for change in dominant frequency, we excluded these notes from the analysis. We excluded the note type that was not reliably classified into subtypes by our Gaussian mixture models from all consistency computations, due to the need to accurately identify subtypes in this analysis.

For each bird, we took the weighted average (or, for spectral cross correlation, the weighted median) across all songs produced by that bird, with each song weighted according to the number of times the note subtype appeared. This produced a value for each consistency measure for each note subtype produced by each bird across all songs that the bird produced. We cannot accurately estimate the variability of a note subtype within songs unless that note subtype is frequently repeated within the same song. Thus, we excluded note subtypes for individual birds if the total number of times the bird produced the note subtype was not greater by at least five than the total number of songs in which the bird produced the note subtype. So, if a bird produced a note subtype in five songs, then the subtype would be included in the analysis if it were produced a minimum of ten times. For the acoustic characteristic variances and the dynamic time warping distance, we log transformed the values to normalise error. For duration, two birds produced one note subtype (out of 420 bird by note subtype combinations in the data) with variabilities more than nine standard deviations below the population mean. These are likely to be errors due to the fact that warbleR measures the durations of notes in discrete units. Therefore, we Winsorized these two values to the smallest observed variability among the other bird by note type combinations in the data.

At this point in the analysis, we had obtained a single value for each of our consistency measures for each note subtype as produced by each bird. However, some note subtypes may be more difficult to produce consistently than others, and individual birds produce different note subtypes. So, to make comparisons among birds, we needed to standardise consistency measures across note subtypes. In our analysis of acoustic characteristics, we standardised across note types produced by different birds by mean centring on each note type. We could do this because note types produced by different birds can be clearly equated. However, note subtypes produced by different birds cannot be clearly equated, so we cannot mean centre at the level of note subtypes. Therefore, we used a modelling approach to control for differences in consistency among note subtypes. We assumed that, within each note type, the consistency of note subtypes might depend on the acoustic characteristic values of the subtype and on the number of times the bird produced the subtype (e.g., if birds learn to produce notes consistently by practicing them more often). For each subtype produced by each bird, we computed the means of the log transformed duration, log transformed mean dominant frequency, and log transformed frequency change, and we counted the number of times the bird produced the subtype and the number of songs in which the bird produced the subtype. We fit our observed consistency values to mixed linear regressions that included fixed effects of every combination of these five predictors, as well as the second and third-order interactions among the acoustic characteristics, and included the note type as a categorical variable. To avoid attributing any effect of individual birds to these predictors (and thus overfitting due to pseudoreplication), we included random effects of the individual bird and the individual bird's natal clutch in the model. We fit models by maximum likelihood using the R package lme4 (Bates et al. 2015). Fitting by maximum likelihood allows us to weight each model according to its Bayesian Information Criterion (Pinheiro and Bates 2000). Then, we computed the model-weighted regression coefficients for each of the predictors we considered in the full model (Burnham & Anderson 2002). Finally, we corrected the observed consistency value for each note in the data set by subtracting the model-weighted fixed effects of its predictors. This left us with a set of residuals that are measures of consistency with the effects of note subtype removed (i.e., with an expected value of zero for every note subtype), but with any random effects of clutch and individual still included in the measure. We computed a single value for each consistency measure for each bird by averaging across all note subtypes that the bird produced.

To ask whether sons learn their consistencies from their social fathers, we regressed the sons' residual consistencies on the residual consistencies of their social fathers for the same consistency measures. We included the sons' log-transformed age in the model, because in many species birds produce notes more consistently as they get older (Kao & Brainard 2006; Botero et al. 2009; De Kort et al. 2009; Rivera-Gutierrez et al. 2010; James & Sakata 2019). We included a random effect of the relatedness matrix in the model to account for potential genetic heritability of consistency, and we included a random effect of natal clutch in the model to account for effects of the rearing environment. If sons learn their consistencies from their social fathers, and if consistency changes with age, then sons are most likely to learn from the consistencies that their social fathers displayed at the time of rearing. In general, the songs of social fathers in our data were not recorded at the time of rearing (mean age at recording 2.64 yrs, sd 1.47 yrs; mean age at rearing of sons 1.87 yrs, sd 1.03 yrs; mean difference 0.76 yrs, sd 1.70 yrs). Therefore, we needed to correct social fathers' consistency measures to reflect their age at time when they were rearing sons. We could not do this by simply including the difference in the fathers' log-transformed ages at the times of recording and rearing sons in the model as a predictor. This would add a free parameter to the model, but in practice the necessary correction for the fathers' consistency is fully determined by the difference in his ages at the times of recording and rearing sons and by the coefficient of log-transformed age in the fitted model. Therefore, we started by fitting models using the social father's uncorrected consistency as a predictor. Then, we corrected the fathers' consistencies using the coefficient of log-transformed age in the model we had just fit, and we refit the model. We repeated this process until the effect of age in the fitted model and the effect of age used in the correction differed by less than a proportion of 10^{-6} of the fitted value. This resulted in models with the fathers' consistency corrected according to the fitted coefficients of the model itself. If the effect of age was not at least marginally significant (i.e., $p>0.1$), we removed log-transformed age from the model and refit, without correcting the fathers' consistency phenotype. We left the social fathers' residual consistency phenotype in the model even if it was not statistically significant. This ensures that we do not incorrectly attribute an effect of learning from the social father to other aspects of the rearing environment simply because the effects of learning are too small to detect with confidence. If there is no learning from the social father, then including the social fathers' phenotypes in the model will incorrectly attribute some clutch effects to learning from the social father, and so reduce the apparent effect of clutch. We assessed the significance of random effects (i.e., relatedness and clutch) using likelihood ratio tests. Likelihood ratio tests for random effects are known to be conservative (Pinheiro et al. 2017).

3.4.5 Ethics statement

This project was reviewed and approved by The University of Manchester Animal Welfare and Ethics Review Board.

3.4.6 Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: FigShare; https://doi. org/10.48420/14555247.

3.5 Results

For the whole song analyses, we found strong evidence that features of song structure are socially learned; son's songs were similar to those of their social father (Table 3.2). For manually assigned note types, there was a strong positive relationship between the social father's and son's songs for all measures with a large associated effect size (all $p<0.001$, Table 3.2, Figure 3.6), i.e., for the structural features measured, sons produced songs with features closely resembling those of their social father. Song structure at the level of computer-assigned note subtypes was also learned (repertoire size, $p<0.0001$; Shannon diversity, $p<0.0001$; first order differential entropy, $p=0.0014$; song linearity, $p=0.0027$; Figure 6), but the effect sizes were generally smaller than those reported for manually-assigned note types.

There was no strong evidence that age of the bird at time of recording influenced any structural measure, although positive trends were found for the number of notes ($p=0.068$), the second order differential entropy (p=0.056) when considering manually assigned note types, and the first order differential entropy (p=0.024) when considering note subtypes. There was no evidence that genes or the rearing environment influenced song structure (Table 3.2). In this analysis, we treated the one note type that was not reliably clustered into subtypes as if it were a single subtype, but our results are qualitatively unchanged if we conduct the same analysis using the original computationally assigned subtypes (Supplementary Information 3.1).

Table 3.2: Results of mixed-effect models for structural features[†] of songs[†].

Response	Social Father's phenotype	log(Age)	Relatedness	Clutch	Relatedness or Clutch
Number of notes*	0.45 p=0.0002	0.12 p=0.068	p=0.11	p=0.15	p=0.10
Note types (manually assigned)					
Repertoire size*	0.82 p<0.0001	0.007 p=0.88	p=0.99	p=0.98	p>0.99
Shannon entropy	0.80 p<0.0001	-0.006 p=0.88	p=0.98	p=0.98	p>0.99
Song linearity	0.39 p=0.0006	0.0046 p=0.54	p=0.89	p=0.10	p=0.27
1st order entropy	0.81 p<0.0001	0.012 p=0.51	p=0.71	p=0.79	p=0.71
2nd order entropy	0.70 p<0.0001	0.035 p=0.056	p=0.97	p=0.94	p>0.99
Note subtypes (computationally assigned)					
Repertoire size*	0.51 p<0.0001	0.095 p=0.14	p>0.99	p=0.17	p=0.40
Shannon entropy	0.50 p<0.0001	0.086 p=0.13	p>0.99	p=0.33	p=0.62
Song linearity	0.37 p=0.0027	-0.015 p=0.11	p=0.99	p=0.98	p>0.99
1st order entropy	0.30 p=0.014	0.060 p=0.024	p=0.83	p>0.99	p=0.98
2nd order entropy	0.13 p=0.33	0.028 p=0.15	p>0.99	p=0.98	p>0.99

*indicates response variable was log-transformed

[†]Across all birds, structural features were computed from a total of 676 songs with a total of 22,972 notes. For each structural feature, we studied data on 58 social father-son pairs.

For the individual note analyses we found strong evidence that acoustic characteristics of note types are learned. For all measures considered, there was a strong positive relationship between the notes produced by social fathers and those of sons (all p<0.001), with large effect sizes (Table 3.3, Figure 3.6); within categories, sons produced notes that were similar to those of their social father. There was no evidence for a relationship between age at time of recording and any of the acoustic characteristics considered (all p>0.1). There was evidence for an effect of clutch for time median (p=0.014), time IQR (p=0.0070) and mean dominant frequency (p=0.041), indicating that birds from the same clutch were more similar than expected by chance alone (Table 3.3). We found no evidence for a genetic effect on any acoustic characteristic (Table 3.3).

Table 3.3: Results of mixed-effect models for acoustic characteristics[†] of notes within songs[†]

Response	**Social Father's phenotype**	**log(Age)**	**Relatedness**	**Clutch**	**Relatedness or Clutch**
Duration*	0.63 p<0.0001	0.040 p=0.67	p>0.99	p=0.99	p=0.86
Time median	0.61 p<0.0001	-0.041 p=0.67	p>0.99	p=0.014	p=0.043
Time IQR	0.60 p<0.0001	-0.080 p=0.43	p>0.99	p=0.0070	p=0.0028
Mean dominant frequency*	0.63 p<0.0001	0.095 p=0.27	p=0.96	p=0.041	p=0.12
Maximum dominant frequency*	0.68 p<0.0001	0.030 p=0.67	p=0.94	p=0.97	p>0.99
Modularity index**	0.53 p<0.0001	-0.13 p=0.18	p=0.82	p>0.99	p=0.98
Frequency change	0.64 p<0.0001	0.12 p=0.13	p>0.99	p=0.54	p=0.83
Peak frequency*	0.62 p<0.0001	0.083 p=0.29	p>0.99	p=0.27	p=0.54

*indicates response variable was log transformed

**indicates response variable was double log transformed

[†] Acoustic characteristics were computed from a total of 20,764 notes, where the note types were produced at least five times by both sons and their social fathers. For each spectral feature, we studied data on 182 social father-son pair x note type combinations.

We found no clear evidence for social learning of vocal consistency (Table 3.4, Figure 3.6), but we found a trend suggesting that social fathers with more consistent note durations had sons with more consistent note durations (p=0.094). Older birds produced note subtypes with more consistent durations than younger birds (p=0.0022). However, there was a trend in the opposite direction for spectral cross correlation – older birds appeared to produce less consistent note subtypes (p=0.066). We found no evidence for an effect of age for any other consistency measure (Table 3.4). There was evidence that the random effects influenced consistency measures in all cases except the variance of frequency change (Table 3.4). For the variance of mean dominant frequency (p=0.0033), the dynamic time warping distance (p=0.0025), and spectral cross correlation (p=0.015), birds from the same clutch were more similar than we would expect by chance alone. For the variance of duration, there was a random effect of either clutch or relatedness (p=0.0056). However, because birds from the same clutch were always genetic brothers in our data, natal clutch and genetic relatedness are correlated, and we cannot determine which of these explains the effect. Visual inspection of the scatterplots (Figure 3.6) revealed three potentially influential points, which were all birds from a single clutch. Results remain qualitatively unchanged if we conduct the same analyses excluding these individuals (Supplementary Information 3.2).

Table 3.4: Results of mixed-effect models for vocal consistency of note subtypes[†]

Response	**Social Father's phenotype**	**log(Age)**	**Relatedness**	**Clutch**	**Relatedness or Clutch**
Note duration*	0.19 p=0.094	-0.34 p=0.0022	p=0.14	p=0.078	p=0.0056
Mean dominant frequency*	0.15 p=0.26	-0.16 p=0.19	p>0.99	p=0.0033	p=0.013
Frequency change	0.098 p=0.51	-0.032 p=0.82	p>0.99	p=0.33	p=0.62
Dynamic time warping	0.059 p=0.64	-0.064 p=0.20	p>0.99	p=0.0025	p=0.010
Spectral cross correlation (median)	0.11 p=0.25	-0.010 p=0.066	p>0.99	p=0.015	p=0.028

[†] Vocal consistency was computed from a total of 18,985 (note duration, mean dominant frequency); 17,917 (frequency change); or 17,808 (dynamic time warping, spectral cross correlation) notes where the same note subtype appeared multiple times in the same song. For each vocal consistency measure, we studied data on 58 social father-son pairs.

*Inspection of scatterplots revealed three potentially influential points, which were all birds from a single clutch. Data were reanalysed with these three birds removed, resulting in changes in significance of some values (see Supplementary Information 3.2), but does not change the interpretation of our results.

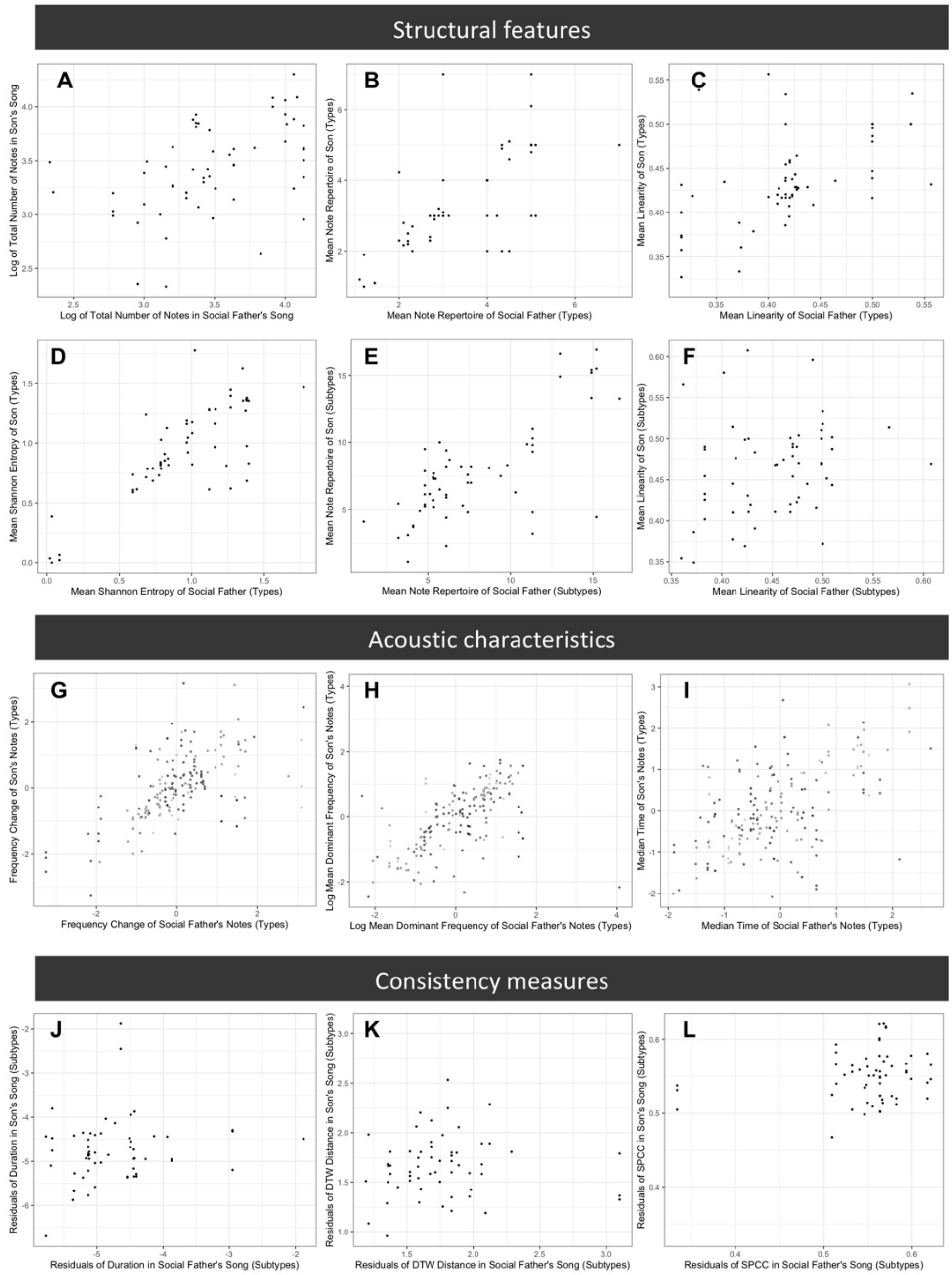


Figure 3.6. Comparison of song features across songs produced by sons and their social fathers. Plots represent a subset of features examined and show typical patterns for each set of features. Plots A-F compare structural features (A) Mean total number of notes in song (song length) B) Mean note type repertoire (manually assigned note types) C) Mean song linearity (manually assigned note types) D) Shannon entropy (manually assigned note types) E) Mean note subtype repertoire (computationally assigned note subtypes) F) Mean song linearity (computationally assigned note subtypes)), G-I compare acoustic characteristics of note types (z-scored), with shading representing different note types (G) Frequency change H) Mean dominant frequency I) Time median, and J-L compare measures of vocal consistency of note subtypes (J) Variance of mean dominant frequency K) Dynamic time warping distance L) Spectral cross correlation

3.6 Discussion

We examined the roles of cultural and genetic inheritance in shaping song phenotypes. There was strong evidence for the social learning of song structure and the acoustic characteristics of notes. Sons’ song structure and note characteristics were similar to those of their social fathers. There were no significant contributions of the genetic pedigree to song phenotype, i.e., birds that were closely related did not have more similar songs when controlling for other factors, and with few exceptions, no effect of age. Here, and throughout, the effect of age may be limited as analyses were confined to the songs of adult birds, where age-related song changes may be slow to manifest or small in magnitude (James & Sakata 2014). For some features, there was an effect of the clutch in which the bird was reared, with individuals from the same clutch more similar than expected by chance alone, indicating an influence of the developmental environment. For vocal consistency, we found no strong evidence of social learning or genetic heritability. However, vocal consistency was influenced by the age of the bird and by the clutch in which the bird was reared, again indicating an influence of the developmental environment. Further empirical work will be required to confirm these patterns in Java sparrows and other species, as our analyses were largely exploratory.

Sons resembled their social father in all measures of song structure, with no effect of genetic relatedness in any case, suggesting that these traits are culturally inherited within populations. Note repertoire size was similar in sons and their social fathers, as is the case for many other species (Grant & Grant 1996; Takahasi & Okanoya 2010; Soma 2011; Labra & Lampe 2018). We also found evidence for cultural inheritance of song complexity, as measured by linearity (Scharff & Nottebohm 1991), and of higher order note sequencing, as measured by differential entropy. Similar sequence learning has been reported recently in Bengalese finch, and birds were more likely to learn note transitions commonly used by their social fathers (James et al. 2020), although we did not examine this in our dataset.

Regression coefficients for the social learning of fathers’ song structure were large, suggesting that there is limited regression towards the population mean in each generation. Thus, sons produced faithful copies of their social fathers’ songs, and differences among song lineages could persist for many generations. When considering note subtypes (i.e., those that were computationally assigned based on clustering of acoustic characteristics), the magnitude of the regression coefficients was smaller. This suggests that regression towards the population mean (as indicated by coefficients closer to zero) is greater when considering note subtypes, and that innovation may be more likely to involve changes among note subtypes than among note types.

Within note types, sons sang notes with similar acoustic characteristics to those of their social fathers. This may mean that birds learn how to produce notes of a particular type from their fathers i.e., they learn the acoustic characteristic values of their social father's note types, or it may mean that they learn which distinct note subtypes to produce from their fathers, which would be reflected in similar mean and variance of note type acoustic characteristics in social father-son pairs.

We found no evidence of genetic inheritance of acoustic characteristics; there was no effect of relatedness on any characteristic measured. This is in contrast to findings from a number of other species (e.g., zebra finch (Forstmeier et al. 2009), Bengalese finch (Kagawa et al. 2014; Mets & Brainard 2018, 2019)) where genetic differences underpin some differences in acoustic characteristics. It is possible that levels of genetic variation within the present laboratory population were not large enough to assess the genetic heritability of acoustic characteristics. Reduced genetic variability in laboratory compared to wild populations has been reported in other species (Forstmeier et al. 2007). In Bengalese finches and white-rumped munia, strain-specific differences in acoustic characteristics of notes are apparent (Kagawa et al. 2014). However, the two strains have high levels of disparity in morphology (Soma 2005) and presumably genetics. A potential caveat of our analysis method (using a relatedness matrix) in determining heritability is that relatedness of founders in our population is unknown. As such, individuals may have been more closely related than suggested by our relatedness matrix.

In contrast to song structure and note acoustic characteristics, we found no strong evidence for the cultural inheritance of vocal consistency, which is a common measure of song performance (but see Supplementary Information 3.2). The vocal consistency of the social father did not predict his sons' vocal consistency. However, contrary to our predictions, we also found no relationship between genetic relatedness and measures of vocal consistency; birds that were related did not have similar levels of vocal consistency. Although evidence was limited, some interesting patterns are apparent. In all models, effect of social father's phenotype was positive, which is consistent with social learning. However, the effect sizes are small in comparison to those for song structure and the acoustic characteristics of notes, so, even if such effects exist, we expect that they will be small. There was some evidence that vocal consistency was related to age at recording; older birds had more consistent note duration across vocalizations than younger birds, although this was not the case for other measures of consistency. Increased vocal consistency with age has been reported in a number of studies across a broad range of bird species, for example (Kao & Brainard 2006; Botero et al. 2009; De Kort et al. 2009; Rivera-Gutierrez et al. 2010). Improved vocal consistency with age may reflect greater opportunity to practice motor patterns involved in vocalizations (Botero & de Kort 2011a; Sakata & Vehrencamp 2012). Thus, if age indicates good genes because the bird has survived or good parental care because the bird has experience, then vocal consistency may be an honest signal of quality in Java sparrows. However, there was no effect of age on the consistency of mean dominant frequency or frequency change for note subtypes within songs, and no effect of age on the dynamic time warping distance among notes of the same subtype. Similarly, James & Sakata (2014) also found no significant age-dependent changes in the mean or variability in a range of frequency-based syllable features in Bengalese finches, although this also included syllable duration. When considering spectral cross correlation within note subtypes, we report a trend in the opposite direction; older birds tended to sing less consistently than younger birds. Variance of note duration and distance by spectral cross correlation are negatively correlated, so, it would be somewhat surprising for either of these patterns to appear by chance alone in the presence of the other. It is therefore not clear how vocal consistency relates to age in male Java sparrows. Our findings suggest the need for further examination of vocal consistency in this species, and for including age in analyses when considering similar questions in other species. There is evidence that differences in consistency are salient (De Kort et al. 2009) and can influence female preference (Woolley & Doupe 2008), male reproductive success (Byers 2007; Cramer et al. 2011) and social status (Botero et al. 2009) in some species. The consistency measures we studied carry information about a singer's age and rearing environment (discussed below). However, determining whether these measures are salient to Java sparrows will require further empirical work.

For a range of measurements across the features examined, we found evidence for an effect of clutch. This reflects an impact of the social father or developmental environment independent of the social father's song. This was especially relevant to vocal consistency, where the majority of measures indicated an important role for the rearing environment. Due to the nature of our dataset, it was not possible to disentangle a number of possible effects, as many factors might contribute to the clutch variable. One possibility is that clutch effects result from differences in the early developmental environment between nests (Nowicki et al. 2002c; Holveck et al. 2008). In the Bengalese finch, birds from larger, male-biased broods had lower song complexity than those from smaller broods (Soma et al. 2006). In this case song features such as consistency may be honest indicators of male quality. Vocal development incurs neural costs during early development (Gil & Gahr 2002) when birds are likely to be exposed to stressors. High quality songs may, therefore, indicate lower stress levels during development (Nowicki et al. 1998; Nowicki & Searcy 2004). As well as increasing developmental stress, large, male-biased broods may also result in fraternal inhibition of song learning. In zebra finches, birds with more male siblings had shorter motif durations and reduced note numbers compared to their tutors (Tchernichovski & Nottebohm 1998). Social reinforcement of learning from parent birds may also play a role in clutch-specific differences in learning accuracy, as parents are likely to show variation in the levels of reinforcement provided. In zebra finches, social feedback from both the father and mother was correlated with song learning, with birds that received appropriate input producing more faithful copies of fathers' songs (Carouso-Peck et al. 2020).

The strong influence of vocal learning, particularly of song structure and the acoustic characteristics of notes, has implications for the evolution and maintenance of song in the Java sparrow. Sons do not precisely copy their social fathers' songs; there are differences, particularly when considering note subtypes, which may relate to improvisation or copying errors during learning. However, we report large effects of the social father's phenotype, suggesting that learning fidelity is high for these traits. In this case, novel variants that arise during the learning process may be preserved and accumulate over generations, contributing to population divergence in song and the formation of vocal dialects (Baker & Cunningham 1985; Catchpole & Slater 2008). Differences in songs among populations, if coupled with female preference for local song types, can result in pre-mating reproductive isolation and, in some cases, speciation (Kirkpatrick 2000; Verzijden et al. 2012). The potential role of song in speciation may be of particular interest in Estrildids, as the family has recently undergone a period of rapid speciation (Olsson & Alström 2020). In a closely related species, the Bengalese finch, female preference for song complexity, coupled with release from selection pressures, has been highlighted as a driver for increasingly complex songs in captivity compared to ancestral wild populations (Okanoya 2012; Suzuki et al. 2014). Whilst the Java sparrow and Bengalese finch share similar life histories and domestication history, little is known about female preference for song features in Java sparrows. It is, therefore, not possible to predict how female mate choice could impact song evolution in the Java sparrow.

The Java sparrow has been widely bred in captivity as part of ex situ conservation efforts (BirdLife International 2018) and as a popular species in aviculture (Restall 1996). Genetic and behavioural change is frequently reported in captive breeding programmes and can accumulate over relatively short time periods, spanning few generations (Håkansson & Jensen 2005; Frankham 2008; Suzuki et al. 2013), and these differences may extend to vocal behaviour (Tanimoto et al. 2017). The potential for song evolution and cultural divergence in the Java sparrow is, therefore, likely to be of interest to conservation practitioners, as vocal changes may influence the success of conservation programmes and in particular reintroduction programmes (Lewis et al. 2020; Crates et al. 2021).

Overall, we find that cultural processes play a large role in the song inheritance of Java sparrow, influencing song structure and complexity, as well as acoustic characteristics of notes, in line with findings in other species. Social inheritance of these features has the potential to influence the formation and maintenance of population specific differences, with implications for evolution and conservation in Java sparrows. However, we found no clear evidence for the inheritance of a performance-related factor, vocal consistency, which was instead related to age at recording and clutch-specific differences. Whilst we did not find a relationship with genetic relatedness, our findings support the hypothesis that vocal consistency is an honest signal of quality, revealing information about the age and developmental environment of the signaller.

3.7 Author Contribution Statement

RL, MS, SdK and TG were responsible for the conception and design of the study. RL and MS collected and collated the data. RL, MS, SdK and TG contributed to the extraction, preparation and analysis of data. The original draft was written by RL. RL and TG contributed to writing sections of the manuscript. All authors were involved in reviewing and editing the manuscript and agreed on the final version.

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3.10 Supplementary Information

Supplementary Information 3.1: Results of mixed effect models for structural features of songs using unedited computational clusters[†]

Response	**Social Father's phenotype**	**log(Age)**	**Pedigree**	**Clutch**	**Pedigree or Clutch**
Number of notes*	0.45 p=0.0002	0.12 p=0.068	p=0.11	p=0.15	p=0.10
Note types (manually assigned)					
Repertoire size*	0.82 p<0.0001	0.007 p=0.88	p=0.99	p=0.98	p>0.99
Shannon entropy	0.80 p<0.0001	-0.0059 p=0.88	p=0.98	p=0.98	p>0.99
Song linearity	0.39 p=0.0006	0.0046 p=0.54	p=0.89	p=0.10	p=0.27
1st order entropy	0.81 p<0.0001	0.012 p=0.51	p=0.71	p=0.79	p=0.71
2nd order entropy	0.70 p<0.0001	0.035 p=0.056	P=0.97	p=0.94	p>0.99
Note subtypes (computationally assigned)					
Repertoire size*	0.61 p<0.0001	0.087 p=0.21	p>0.99	p=0.29	p=0.57
Shannon entropy	0.63 p<0.0001	0.057 p=0.39	p>0.99	p=0.39	p=0.69
Song linearity	0.23 p=0.11	-0.011 p=0.25	p=0.98	p=0.98	p>0.99
1st order entropy	0.30 p=0.017	0.051 p=0.067	p=0.84	P>0.99	p=0.96
2nd order entropy	0.10 p=0.49	0.030 p=0.13	p=0.99	P>0.99	p>0.99

*indicates response variable was log-transformed

[†]Across all birds, structural features were computed from a total of 676 songs with a total of 22,972 notes. For each structural feature, we studied data on 58 social father-son pairs.

Supplementary Information 3.2: Results of mixed-effect models for vocal consistency without one clutch that appeared influential in scatterplots[†]

Response	**Social Father's phenotype**	**log(Age)**	**Relatedness**	**Clutch**	**Relatedness or Clutch**
Note duration	0.16 p=0.23	-0.34 p=0.0022	p=0.15	p=0.30	p=0.042
Mean dominant frequency	0.34 p=0.033	-0.17 p=0.15	p>0.99	p=0.036	p=0.11
Frequency change	0.095 p=0.52	-0.071 p=0.62	p>0.99	p=0.60	p=0.87
Dynamic time warping	0.25 p=0.17	-0.055 p=0.28	p>0.99	p=0.0049	p=0.019
Spectral cross correlation (median)	0.11 p=0.25	-0.010 p=0.067	p>0.99	p=0.020	p=0.039

[†] Vocal consistency was computed from a total of 17,594 (note duration, mean dominant frequency); 17,011 (frequency change); or 16,786 (dynamic time warping, spectral cross correlation) notes where the same note subtype appeared multiple times in the same song. For each vocal consistency measure, we studied data on 55 social father-son pairs.

Chapter 4: Inheritance of temporal song features in Java sparrows

Rebecca N Lewis, Anthony Kwong, Masayo Soma, Selvino R de Kort, R Tucker Gilman

4.1 Chapter Summary

In Chapter 3, I discussed the inheritance of three categories of song features in the Java sparrow: song structure, note acoustic parameters and note consistency. Whilst these three categories are often considered in the study of birdsong, there are many other components of song that are less well-studied. One such component is the temporal features of the song, relating to timing and tempo. These features appear salient to females, and preference for fast or long duration songs has been reported in some species (Nolan & Hill 2004; Dunning et al. 2020), suggesting they may have a role in mate choice. Whilst temporal features have been frequently examined with respect to age (James & Sakata 2014, 2015, 2019) and context (James & Sakata 2015, 2019; James et al. 2018), the inheritance of these features has received comparatively less attention than other components of song in estrildid finches, although has recently been examined in Bengalese finches (Mets & Brainard 2018, 2019) and zebra finches (James et al. 2022).

There are a number of possible modes of inheritance for temporal song features. Firstly, as with other song features, they may be learned socially from a tutor. This is likely to be important, as birds without tutors often develop atypical temporal patterning (Marler & Sherman 1985; Feher et al. 2009; Kagawa et al. 2014). However, due to constraints in vocal production, apparent learning of temporal features may be a by-product of vocal production learning. Finally, as with other song factors, genetic background and the developmental environment may also influence temporal features.

In this chapter, I examine the inheritance of temporal song features using the dataset described in Chapter 3. We selected a suite of temporal features, many of which are commonly used across the literature e.g., tempo, duration, gap length duration. We also included the duration of shared gaps, i.e., gaps between note sequences that appear in both the son's and social father's song. Comparison of shared gaps has recently been used as a metric in zebra finches, so its inclusion allows for a more direct comparison between the two species. We also used two more complex metrics, which aim to assess a bird's vocal performance compared to the rest of the population, whilst taking into account among individual variation in song. As in Chapter 3, I examine the relative contributions of social learning, genetic inheritance, and the developmental environment. Whilst the learning of some temporal song features has been assessed, to our knowledge, this is the first study to examine the contributions of different modes of inheritance across a broad range of temporal song features.

4.2 Abstract

The inheritance of structural characteristics and individual note parameters is often studied in oscine passerines. However, other song features, such as the temporal characteristics of song, are less well studied. Inheritance of these features could be influenced by a range of factors, including social inheritance, mechanical constraints, genetic relatedness, and the developmental environment. Using archived song data, we examined patterns of inheritance of temporal song features, relating to song duration, song tempo, and inter-note intervals, in a laboratory population of Java sparrows (*Lonchura oryzivora*), some of which had been cross fostered. Overall, we found strong evidence for cultural inheritance of temporal song features, i.e., temporal song features were learned from the social father. We found that duration was, at most, weakly socially inherited and constrained by a correlation between song tempo and number of notes. This suggests that song duration may be hard to increase, and highlights its potential as an honest indicator in this species. Across all measures, we found no evidence that temporal song features were affected by genetic relatedness or the developmental environment. Our findings suggest that social learning is the main mode of inheritance for temporal song features in the Java sparrow. This may be caused by the specific learning of temporal song features or as a by-product of learning of note repertoire and song structure. It is likely that learning of temporal features interacts with vocal learning in the development of the adult song phenotype, and the relationship between these two components could be further examined through experimental studies.

4.3 Introduction

Bird song is a complex social signal with an important role in courtship and mate choice. The inheritance of birdsong structure (e.g., note repertoire or structural complexity) and specific note parameters (e.g., frequency, duration and amplitude) are frequently studied. These features can be honest indicators of male quality (Gil & Gahr 2002) and can shape female preferences (Collins 2004; Ritschard et al. 2010) and thus sexual selection (Ryan 1997). A range of mechanisms, including social learning (Beecher & Brenowitz 2005; Lewis et al. 2021a), genetic inheritance (Forstmeier et al. 2009), motivation (Leedale et al. 2015), and developmental stress (Nowicki & Searcy, 2004), can influence song development and adult song phenotype. Understanding the contribution of these different components to adult song phenotype is therefore vital to making predictions about song evolution over time.

Many of the same principles apply to the temporal characteristics of song, such as tempo (which we define as the note rate) and inter-note intervals. However, temporal features are particularly interesting, as they often show more within- and between-individual variation than other song features (Glaze & Troyer 2006). In both zebra finches (*Taeniopygia guttata*) (Glaze & Troyer 2006) and Bengalese finches (*Lonchura striata domestica*) (Tachibana et al. 2015), the variation in inter-note interval was considerably greater than that of note lengths. Temporal features are salient to females, and females show preference for songs with particular temporal characteristics (Nolan & Hill 2004; Dunning et al. 2020). Although temporal song features could contribute to sexual selection, their inheritance has received limited attention compared to other song features.

Temporal features may be learned from a tutor. Untutored birds can show unusual song timing (e.g., Kagawa et al., 2014), suggesting that a tutor may be necessary in developing species-specific temporal features. Zebra finches show no significant difference in duration between fathers and sons for identical note-to-note transitions (James et al. 2022). This pattern was also seen when birds were experimentally tutored with songs that had the same gap durations for all note-to-note transitions (James et al. 2022), suggesting that gap duration may have a learned component which is separate from the notes themselves. However, temporal features can also be mechanistically linked to other song features. In Bengalese finches, individuals with larger note repertoires sang slower songs, suggesting a trade-off between repertoire size and tempo (Soma et al. 2006a). Note repertoire can be socially inherited (Soma 2011; Lewis et al. 2021a), so learning of note repertoire may result in similar tempos between fathers and sons due to a shared repertoire and repertoire size. In Bengalese finches, the length of silent gaps was significantly correlated with the length of the preceding note (Tachibana et al. 2015). Gap duration was also affected by the note-to-note transition; stereotyped transitions, which are the same each time they are sung, were significantly shorter than branching points, where a syllable may be followed by more than one different syllable types (Matheson & Sakata 2015; Tachibana et al. 2015). Specific note-to-note transitions and branching points are often shared between fathers and sons (James et al. 2020). Whilst the duration of silent gaps could be learned directly from the tutor's song, it is possible that they are learned indirectly through the learning of note repertoires and sequences. As such, apparent learning of a broad range of temporal features may simply be a by-product of the learning of note types and song structures. If inheritance of temporal features is related to vocal learning, we would expect to see clear signal of social learning (similarity between social fathers and sons) for shared note-to-note transitions. However, if learning of temporal features is independent to vocal learning, we would expect to find evidence of social learning across songs, not just for shared transition types.

Genetic background can also influence temporal song features. In Bengalese finches, genetic background influenced the fidelity of learning for temporal features; learning of tempo was more accurate when the tutor's song more closely matched the genetic background of the individual i.e., was more similar to the tempo of their genetic father's song (Mets & Brainard 2018, 2019). Temporal features may also be affected by morphological characteristics. Beak shape influences temporal song features in a range of clades (Derryberry et al., 2012, 2018; Friedman et al., 2019;

García & Tubaro, 2018) and within individual species (Podos 2001; Huber & Podos 2006). In general, birds with larger beaks produced songs with slower tempo (Derryberry et al. 2012, 2018; García & Tubaro 2018; Friedman et al. 2019) and longer silent intervals between notes (García & Tubaro 2018). These influences of morphological characteristics are likely to contribute to a genetic component of the inheritance of temporal song features. If genetic background influences temporal song features, we would expect to see correlations in traits between related individuals, even if birds were not raised by their genetic fathers.

Temporal song features may also indicate male quality, suggesting they could be honest indicators. The syllable rate of zebra finch nestlings with food restricted parents was lower than that of those without restrictions (Zann & Cash 2008), suggesting that temporal song features could indicate early developmental stress. If the developmental environment plays a role in shaping temporal song features, we would expect to see correlations in traits among individuals with shared developmental history i.e., clutch mates. Song tempo is also positively correlated with age in a number of estrildid finch species (Glaze & Troyer 2013; James & Sakata 2014, 2015, 2019; Ota & Soma 2014), whereas gap length and age appear to be negatively correlated (Glaze & Troyer 2013; James & Sakata 2014, 2015). There is a positive relationship between age and male quality through selective attrition. Temporal song features could, therefore, be used as a basis for males to advertise individual quality and to inform female mate choice.

Temporal features can play a role during courtship and mate choice. Female Bengalese finches showed a preference for songs with higher tempo (Dunning et al. 2020). The importance of temporal features is further highlighted when comparing undirected vs. female directed songs. Bengalese finches increase song tempo and decrease inter-note intervals when performing female-directed, rather than undirected song (James & Sakata 2015; Matheson & Sakata 2015). Female directed songs also have higher levels of consistency (Sakata et al. 2008). In addition, in blue tits, where both sexes sing, males sing with higher consistency than females, and consistency increased towards the breeding season (Sierro et al. 2022), suggesting that consistency is important for mate choice. As such, temporal features may be important in sexual selection, especially if they reveal information about the singer's learning ability, genetic background, developmental environment, or individual traits.

Estrildid finches, particularly zebra finches and Bengalese finches, are frequently used as a model group for the study of song development and inheritance. We studied patterns of inheritance of temporal song features in another estrildid species, the Java sparrow (*Lonchura oryzivora*), an endangered finch native to South East Asia (BirdLife International 2018). We used the same dataset as Lewis et al. (2021a), which contains song recordings from Java sparrows over multiple generations. Some birds were raised by their genetic fathers, whilst others were cross-fostered and raised by social fathers that were not their genetic fathers. As such, this dataset is an excellent tool for disentangling social learning and genetic heritability. We examined the dataset for evidence of social learning, genetic heritability, and rearing environment on the inheritance of temporal song features. We expected that temporal song features would be socially inherited, as is the case with other song features in this species (Lewis et al. 2021a), but with potential contributions from genetic inheritance and the developmental environment.

4.4 Methods

4.4.1 Study Species

The Java sparrow is an estrildid finch native to South East Asia and frequently kept in captivity (Restall 1996; BirdLife International 2018). Each juvenile male learns to sing a single song type during a critical period (Ota & Soma 2014), and, as adults, males use these songs for courtship. Song learning requires social interaction with the tutor, so in laboratory settings juveniles that do not interact with other males are most likely to learn from their social fathers (Soma 2011). Previous studies suggest that a range of song features, such as song structure and note characteristics, are inherited from the social father and not related to the genetic pedigree (Lewis et al. 2021a). However, spectrogram inspection suggests that copy accuracy of the temporal features of songs, such as the duration of inter-note intervals, may be lower than that of other song features (Soma 2011). The relative contributions of cultural and genetic inheritance, as well as rearing environment, to a wide range of temporal song features has not, to our knowledge, been formally assessed in this species. See Appendix 1 for further details on subjects and housing.

4.4.2 Dataset

To investigate the inheritance of temporal song features, we used the dataset from Lewis et al. (2021a). This dataset contains information on individual notes in 676 undirected songs from 73 Java sparrows across multiple generations with known social and genetic pedigrees (see Lewis et al., 2021a). In total, 58 father-son pairs are present in the dataset. Sons were raised by their genetic fathers (28) or by social fathers that were not their genetic fathers (30). See Lewis et al. (2021a) for further details on dataset construction.

Songs were defined as a series of notes with inter-note intervals of <1s (Kagawa & Soma 2013; Lewis et al. 2021a). Songs were manually segmented into individual notes using Koe (Fukuzawa et al. 2020) (window length = 512, window overlap = 50%, time-axis zoom = 400%, contrast = 100%) (Lewis et al., 2021a). In total, 22,972 notes were classified into 16 note types based on a suite of characteristics (presence of harmonics, frequency modulation, length, and presence of non-linear phenomena). Classification was repeatable across observers (Lewis et al. 2021a). We cleaned the original dataset to remove four notes separated from their songs by inter-note intervals of >1 s. In addition, we added two notes (from a single song) that were not included in the dataset despite an inter-note interval of <1 s. These two notes were segmented and classified as in Lewis et al., (2021a). The final dataset used for the present study included 22,970 notes from 676 songs and 73 individuals (mean 9.3 songs per individual, range 3-10).

4.4.3 Measurement of song features

4.4.3.1 Identification of introductory notes

Songs frequently contained series of introductory notes. These notes may represent a period of preparation prior to the main song, as reported in zebra finches (Rajan & Doupe 2013; Rao et al. 2019; Kalra et al. 2021). In zebra finches, acoustic structure and the number of introductory notes before the main body of the song is learned from a tutor, but also shaped by biological predispositions (Kalra et al. 2021). This suggests that these notes are not solely related to preparation, and may be important in song learning and development and thus require investigation. This does, however, create some problems when considering temporal features of songs. Introductory sections were more variable than the main bodies of songs and were characterised by relatively long silent intervals between notes (>0.25 s but <1 s). Although in other species, these introductory notes appear to be structurally distinct from the main song motif (Rajan & Doupe 2013; Rao et al. 2019; Kalra et al. 2021), in Java sparrows, introductory notes were structurally similar to notes found elsewhere in the song, making them harder to define. The presence and number of introductory notes was highly variable both within and between individuals. As such, the presence of introductions in songs may influence our estimates of inheritance. For example, if a son generally uses introductory notes, but his social father does not, the son’s gap lengths and standard deviation will be inflated compared to that of his father, potentially masking any pattern of inheritance in the main body of the songs. In Java sparrows, whilst a 1-second inter-note-interval is often used to describe songs (Kagawa & Soma 2013; Lewis et al. 2021a), other studies have used a smaller inter-note-interval for inclusion to exclude introductory notes (Ota & Soma 2014). To account for the presence of introductory notes, all analyses were conducted twice – once with and once without introductory notes - to determine if patterns were still apparent when these notes were excluded.

To remove introductory notes, we reduced the inter-note interval for inclusion in the song to 0.25 s, rather than 1 s. However, birds would sometimes have gaps of this length in the middle of their main songs, and birds often sang more than one introductory note in quick succession followed by a long gap. To account for these features, we considered the introduction to have ended once five consecutive notes with inter-note interval of less than 0.25 s had been identified (Figure 4.1)

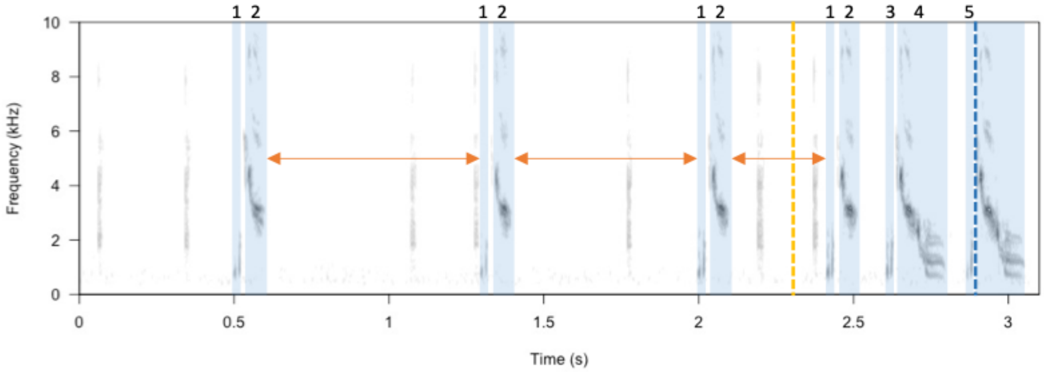

Figure 4.1: Illustration of the identification of introductory notes. Orange arrows represent inter-note-intervals larger than 0.25s. The note count resets each time a gap >0.25s is identified. Once a fifth consecutive note with a gap of <0.25s was identified, we no longer searched the songs for intervals >0.25s (blue dotted line). The start of the main song is identified as the point at which the string of consecutive notes started (yellow dotted line)

4.4.3.2 Temporal song features

Temporal song features were extracted from the processed dataset, which contained information on the start time, end time, and duration of notes in each song. We defined gaps to be the period of silence between the end of one note and the beginning of the next (inter-note interval). Gaps were assigned a transition type based on the immediately preceding and following notes. We focused on six temporal features: 1) mean song duration (s): average song length for each individual; 2) song tempo (notes per second): The mean number of notes produced per second by an individual across all of its recordings; 3) mean gap length (s): average logged gap duration across all of an individual's recordings; 4) gap length standard deviation (s): standard deviation of the logged gap duration across all of an individual's recordings; 5) gap score: a measure of vocal performance comparing an individual's gap lengths to the gap lengths of the rest of the population for the same transition types. For each transition type, we computed the deviation of the individual's mean logged gap length for that transition type from the population mean logged gap length for the same transition type. The gap score of the individual is the weighted mean of the deviations across all its transition types. The motivation is that gap length has been observed to be influenced by transition type in other species (Matheson & Sakata 2015). We designed this statistic to investigate whether social fathers who sing longer gaps also produce sons who sing longer gaps, whilst controlling for potential differences in transition types between social fathers and their sons; 6) gap variability score: a measure of consistency across gaps in an individual's songs, controlling for transition types. We computed the coefficient of variation, c_{jb}, for the logged gap duration of each transition type j as produced by each bird b. Then, we computed the weighted population mean coefficient of variation, c_j, for each transition type j in the population. Thus, $c_j =$

$\sum c_{jb}(n_{jb}-1)/\sum(n_{jb}-1)$ where n_{jb} is the number of times bird b produced transition type j and the summations run over all birds that produced transition type j at least twice. Then, the variability score for bird b producing transition type j is $z_{jb}=\log(c_{jb}/c_j)$, and the gap variability score for bird b is $z_b=\sum z_{jb}(n_{jb}-1)/\sum(n_{jb}-1)$ where the summations run over all transition types that bird b produced at least twice. Similar to the gap score, this statistic enables us to investigate gap length variability whilst controlling for differences in variability among transition types.

In addition, we studied the social learning of gap lengths for transition types that were shared between sons and their social fathers (i.e., only those transitions that appear in both the song of the son and his social father).

4.4.4 Data analysis

We investigated the potential social and genetic inheritance of the six temporal features using linear mixed effects models fitted with the lmekin function from the coxme R package, which accounts for the genetic pedigree via a relatedness matrix (Therneau 2018). For each feature, we regressed the son's phenotype against the social father's phenotype and the log age of the son at the time of recording. Age was included because Java sparrow songs have been observed to change with age after the crystallization period (Ota & Soma 2014). As fathers were not always recorded at the age when they raised sons, and due to the possible effects of age on temporal features (James & Sakata 2014, 2015, 2019; Ota & Soma 2014), we corrected the social father's phenotype to represent his phenotype when rearing sons using the methods described in Lewis et al. (2021a). Briefly, we fit a model to the raw data and then adjusted the social father's phenotype by subtracting the difference in his log age at the time of recording and the time at which the son was reared, multiplied by the fitted coefficient for log age. We repeated this process in a loop which terminated when the fitted coefficient for log age changed by less that 10^{-4} between iterations. Clutch and the genetic pedigree were included in the model as random effects. We modelled the genetic pedigree using a relatedness matrix produced by the kinship2 R package (Sinnwell et al. 2014). Using a relatedness matrix, rather than only information from genetic fathers, allows us to use information about more distantly related individuals, which increases our ability to detect genetic effects in the dataset. Clutch was included to account for the shared environment between nestmates. To test whether the clutch and the genetic pedigree, or either of the two factors respectively, affect the temporal features of Java sparrow song, we fitted reduced models with those random effects removed. We compared the reduced models with the full model using likelihood ratio tests. To examine the inheritance of the length of transitions that were sung by both sons and their social fathers, we regressed sons' mean logged gap duration on social father's logged mean gap duration for the same transition type. We then fit the same model described above but with the additional random effects of transition type and individual bird. The random effect of transition type accounts for the fact that different transition types have different population mean lengths, and the random effect of individual accounts for the fact that some birds may have systematically longer or shorter gap lengths than others. As we were also interested in the effect of introductions on temporal features, the above tests were repeated with the introductory notes removed from all songs. We also repeated the above test to examine the patterns of inheritance for the number of introductory notes, which were logged prior to testing.

4.5 Results

Different transition types had different mean gap durations (Supplementary Information 4.1). Among transition types that were observed more than 10 times, the mean gap length ranges from 0.019 s to 0.156 s.

When considering full songs, we found evidence for social inheritance in all traits measured except song duration and gap score (Table 4.1; Figure 4.2). That is, birds' temporal song features were more similar to the song features of their social fathers than to those of other birds. When introductions were removed, patterns remained consistent (Table 4.2; Figure 4.3), with the exception of song duration, which showed evidence of social inheritance (Table 4.2). Across all features measured, both in full songs and songs without introductory notes, there was no evidence for an effect of genetic inheritance (pedigree) on temporal features of songs (Table 4.1; Table 4.2; Figure 4.2: Figure 4.3). Thus, birds' song features were not significantly correlated with the song features of their genetic relatives. Similarly, there was no evidence for an effect of the developmental environment (clutch) on temporal features (Table 4.1; Table 4.2; Figure 4.2; Figure 4.3).

Table 4.1: Results for mixed effect models on whole songs. * indicates values were log transformed for inclusion in the model. Significant results are indicated in bold.

Response	**Social Father's phenotype**	**log(Age)**	**Pedigree**	**Clutch**	**Either**
Song duration	0.19 p=0.181	**0.76** **p=0.019**	p=0.172	p=0.319	p=0.105
Song Tempo	**0.62** **p<0.001**	-0.049 p=0.169	p=0.255	p=1	p=0.510
Mean gap length	**0.65** **p<0.001**	-0.0094 p=0.874	p=0.399	p=1	p=0.701
Gap length for shared transitions	**0.73** **p<0.001**	0.00024 p=0.98	p=1	p=1	p=1
Gap length standard deviation	**0.54** **p<0.001**	0.052 p=0.170	p=1	p=0.121	p=0.301
Gap score	0.063 p=0.662	-0.022 p=0.478	p=0.380	p=1	p=0.681
Gap length variability score	**0.38** **p=0.008**	0.075 p=0.458	p=0.612	p=0.615	p=0.635
Number of introductory notes*	-0.0038 p=0.980	**0.39** **p=0.008**	p=0.290	p=0.431	p=0.219

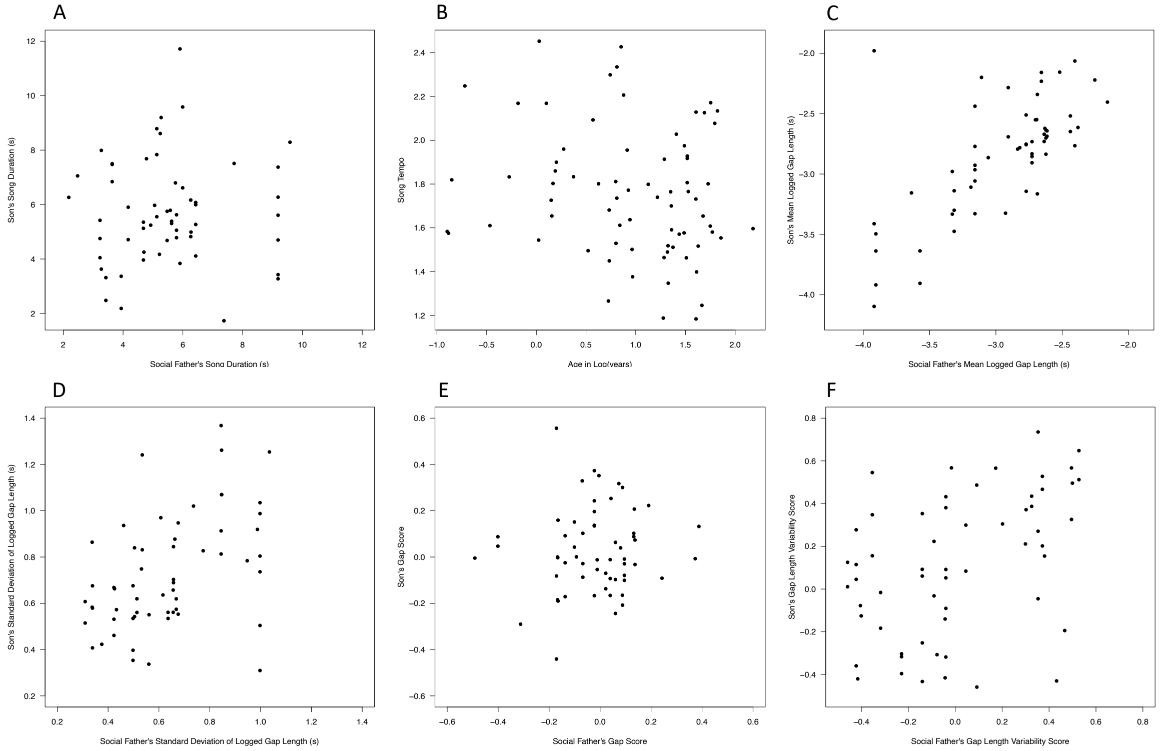

Figure 4.2: Relationships between temporal song features of the social father compared to characteristics of the son in full songs A) Song duration B) Song tempo C) Mean gap length D) Gap length standard deviation E) Gap score F) Gap length variability score. Each point represents the mean value of an individual's characteristics across their songs in the dataset (n=58 sons)

Evidence for age effects was more variable. When considering full songs we found an effect of age on song duration, with older birds singing longer songs (Table 4.1; Figure 4.4A). However, the patterns showed some differences when considering songs without introductory notes. Firstly, whilst the effect is in the same direction, there was no longer a significant effect of age on song duration (Table 4.2; Figure 4.4D) We did, however, find a significant effect of age on the gap score: older birds had shorter inter-note intervals when controlling for the transition type (Table 4.2; Figure 4.4F).

Table 4.2: Results for mixed effect models on songs with introductory phrases removed. Significant results are indicated in bold.

Response	**Social Father's phenotype**	**log(Age)**	**Pedigree**	**Clutch**	**Either**
Song duration	**0.27** **p=0.044**	0.29 p=0.180	p=0.175	p=0.219	p=0.071
Song Tempo	**0.65** **p<0.001**	-0.0065 p=0.817	p=0.231	p=1.000	p=0.489
Mean gap length	**0.64** **p<0.001**	-0.041 p=0.43	p=0.427	p=1.000	p=0.730
Gap length for shared transitions	**0.80** **p<0.001**	-0.0072 p=0.56	p=1.000	p=1.000	p=1.000
Gap length standard deviation	**0.80** **p<0.001**	0.019 p=0.55	p=1	p=0.487	p=0.786
Gap score	0.010 p=0.94	**-0.053** **p=0.05**	p=0.392	p=1.000	p=0.694
Gap length variability score	**0.33** **p=0.018**	0.0084 p=0.931	p=0.684	p=0.337	p=0.524

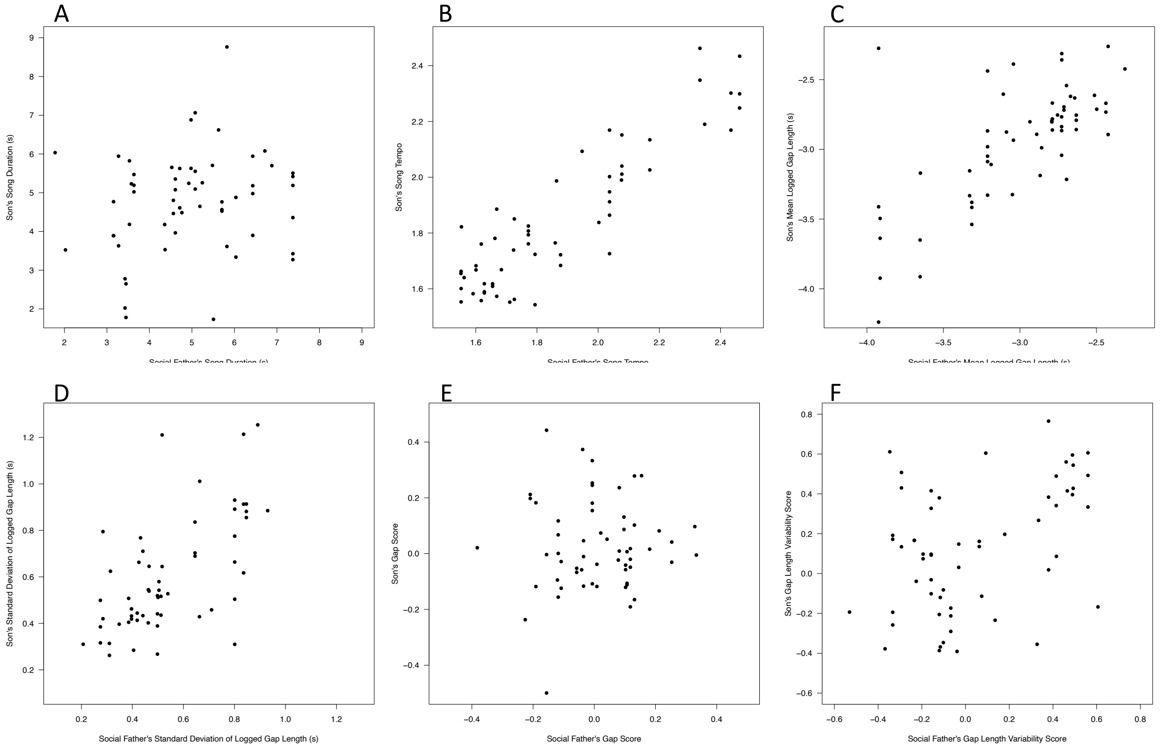

Figure 4.3: Relationships between temporal song features of the social father compared to characteristics of the son in songs with introductory notes removed A) Song duration B) Song tempo C) Mean gap length D) Gap length standard deviation E) Gap score F) Gap length variability score. Each point represents the mean value of an individual's characteristics across their songs in the dataset (n=58 sons)

Since both patterns relating to age and inheritance were influenced by the removal of the introduction, we further examined the removed introductory phrases. We found no evidence that the number of introductory notes was socially inherited; sons with more introductory notes did not have social fathers with more introductory notes (Table 4.1). However, the number of introductory notes changed with age, with older birds having more introductory notes (Table 4.1; Figure 4.4C).

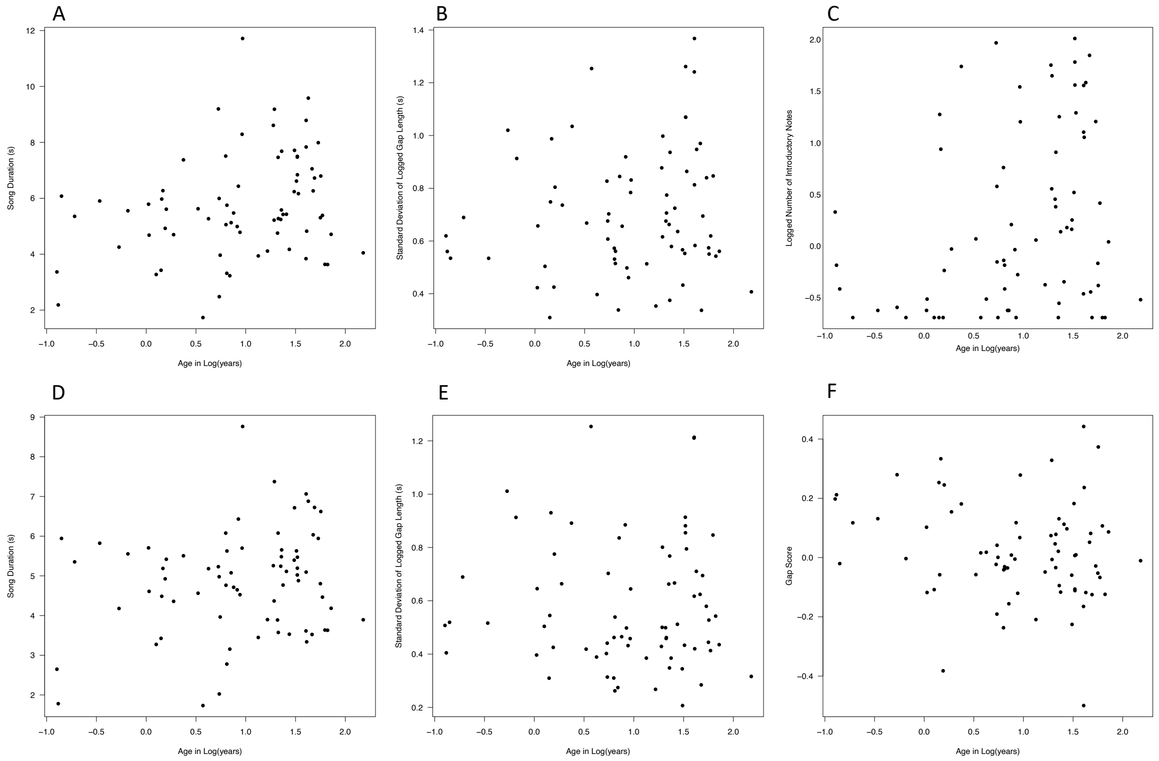

Figure 4.4: Relationship between bird's age at recording and song characteristics A) Song duration of the full song B) Gap length standard deviation in the full song C) Log of the number of introductory notes D) Song duration with introductory notes removed E) Gap length standard deviation with introductory notes removed F) Gap score with introductory notes removed. Each point represents the mean value of an individual's characteristics across their songs in the dataset (n=73 birds)

4.6 Discussion

We found strong evidence that temporal features of birds' songs were socially inherited. There was no evidence that temporal song features were genetically inherited or affected by the developmental environment.

Most temporal features in songs showed strong evidence of social inheritance, both in full songs and in songs with introductory phrases removed. That is, an individual's song features correlated strongly with those of their social father. Similar results were found in the closely related Bengalese finch when raised with a live tutor present (Mets & Brainard 2018). In Bengalese finches, larger note repertoires (more note types) were associated with slower song tempo (Soma et al. 2006b). Note repertoire and the number of note types are socially inherited in Java sparrows

(Soma 2011; Ota & Soma 2014; Lewis et al. 2021a), so repertoire learning may contribute to the learning of song tempo due to matching of note types, trade-offs, and mechanical constraints. Across songs, mean gap length was similar between sons and their social fathers, suggesting that gaps were socially learned. However, we found a particularly strong effect of social inheritance for gap lengths of shared transitions between social fathers and their sons, suggesting that, due to mechanical constraints of note production, the learning of note characteristics may drive similarities between the gap lengths of fathers and sons. In zebra finches, a similar pattern was found: gap lengths for shared note type transitions were correlated between fathers and sons in live-tutored birds (James et al. 2022). However, the study also used experimental tutoring to further investigate inheritance. Birds tutored with artificial songs that had uniform gap lengths also had gap lengths that were similar to the tutor stimulus. This suggests that social learning of gap length is at least partially independent from vocal learning, as son's gap length was not strongly influenced by the preceding note type. It seems likely that a similar pattern could occur in Java sparrows, although this would need to be tested experimentally. The importance of a tutor is also highlighted by the presence of longer inter-note intervals in untutored compared to tutored Bengalese finches (Kagawa et al. 2014). Although mean and shared gap lengths were socially inherited, we found no evidence of social inheritance for gap score, which quantifies gap lengths controlled for transition type compared to the rest of the population. That is, social fathers with longer average gap lengths across all transition types did not raise sons with longer average gap lengths across all transition types. Such a pattern may be apparent if birds do not consistently sing longer- or shorter-than-average gap lengths, instead having a mixture of relative gap lengths. We found that a measure of consistency, the gap variability score, was strongly socially inherited. Variability for shared transitions was not correlated between fathers and sons in zebra finches (James et al. 2022). However, our measure takes into account that different father-son pairs may sing different transitions, and that some of these transitions may be inherently more variable than others (e.g., due to length or difficulty). Taken together, this study, along with studies in other birds, suggests that specific learning of temporal song features, such as inter-note interval, interacts with vocal learning to contribute to the adult song phenotype.

We did not find strong evidence for social inheritance of song duration. At first, this seems surprising, since gap length, note duration (within note types) and song structural characteristics, such as number of notes and complexity, are also socially inherited (Lewis et al. 2021a). We might, therefore, expect song duration to be socially inherited. Song duration is determined by the number of notes in a song and by the song tempo. However, in our data, these two features are correlated; birds with more notes in their song sing them at faster tempos. In this way, it appears that duration is partially constrained, and birds cannot simply double their song duration by doubling the number of notes they sing. This suggests that song duration could act as an honest indicator of male quality, as it may be relatively difficult to change. Singing long songs is energetically challenging (Gil & Gahr 2002), so energetic constraints may contribute to its role as an honest indicator. In line with this, female house finches (*Carpodacus mexicanus*) showed a preference for long over short duration songs, indicating that variation in duration is salient to females and may be used in mate choice (Nolan & Hill 2004). The differences between songs with and without introductory notes is likely related to the characteristics of introductions. Introductions are characterised by long inter-note intervals, so can strongly impact overall song duration. However, the number of introductory notes was not socially heritable, which could contribute to differences between sons and their fathers.

Contrary to our predictions, we found no evidence that genetic pedigree influenced temporal features in Java sparrow song. Consistent with this finding, other song features in Java sparrows, specifically those relating to overall song and note structure, also showed no evidence of genetic inheritance (Lewis et al. 2021a). This is contrary to findings in other estrildid finch species. In Bengalese finches, there was an interaction between learning environment and genetic background for song tempo (Mets & Brainard 2018, 2019). Birds tutored with identical songs produced songs with tempos correlated with those of their genetic fathers (Mets & Brainard 2018) and computer tutored birds learned more accurately if song tempo was more similar to that of their genetic fathers (Mets & Brainard 2019). However, when birds were live-tutored, the relative contribution of genetics to song phenotype was dramatically reduced, whilst the relative contribution of the tutor's song increased, suggesting that live tutoring may overcome genetic contributions to song temporal features (Mets & Brainard 2018). Given the findings in Bengalese finches, and as birds in our dataset were live-tutored, it is possible that the relative contribution of genetic inheritance was reduced to a point where it was not detectable in our analyses, although this seems unlikely. It is also possible that genetic variation in this population was too small to reliably assess genetic inheritance, especially as the relationships between founding individuals were not known.

We also found no effect of the developmental environment (clutch) on temporal features. The developmental environment captures attributes such as provisioning, brood size, and parental feedback during song learning (Carouso-Peck et al. 2020). Previous studies in Java sparrows found that note consistency, but not song or note structure, was affected by an individual's clutch (Lewis et al. 2021a). Our ability to detect clutch effects in this study may have been limited as all clutches were raised in captivity in relatively similar conditions, and there may have been variability within clutches due to asynchronous hatching (Soma et al., 2009). In zebra finches, higher developmental stress (restricted foraging (Zann & Cash 2008) and clutch size (Holveck et al. 2008)) affected temporal song features. However, developmental stress did not affect song speed in Bengalese finches, but larger birds (which were generally from smaller clutches) produced song bouts with longer durations (Soma et al. 2006b). Further research and experimental manipulation are likely needed to fully understand the effect of the developmental environment on song phenotype in Java sparrows.

We did not find strong evidence that temporal features were affected by age at recording, although we found some effect of age on song features in certain scenarios. Changes in song characteristics with age have been frequently reported across birds (Kipper & Kiefer 2010). Delayed maturation of song characteristics may result in song characteristics being an honest signal of age (De Kort et al. 2009; Zipple et al. 2019; Kochvar et al. 2022). This can signal male quality, as older males have demonstrated an increased survival ability (Kokko 1998). An increase in song duration with age has previously been reported in Java sparrows (Ota & Soma 2014). We also find song duration increased with age when introductory notes were included, although, when introductory notes were removed, the effect was in the same direction but it was not significant. A larger effect in whole songs is likely due to an increase in the number of introductory notes with age. However, an increase in song duration with age further highlights its potential as an honest indicator. Previous studies in estrildid finches found an increase in individual birds' song tempo with age (Java sparrows (Ota & Soma 2014), Bengalese finches (James & Sakata 2014, 2015), zebra finches (Glaze & Troyer 2013; James & Sakata 2019)). Contrary to these findings, we did not find an effect of age on song tempo. In Bengalese finches (James & Sakata 2014, 2015; Matheson & Sakata 2015) and zebra finches (Glaze & Troyer 2013), the duration of gaps between notes also decreased with age, which contributed to increased tempo. We found no decrease in mean gap length with age, suggesting that, on average, gap duration did not decrease. However, there was some evidence that gap score, a performance-related measure examining gap length across transitions compared to the rest of the population, decreased with age when considering the main body of the song (no introductory notes), suggesting an increase in performance with age. For whole songs, the non-significant effect was in the same direction, but may have been masked by an increase in introductory notes with age. However, another performance measure, gap variability score, which relates to consistency, did not change with age. Although variability of gaps decreased with age in Bengalese finches (James & Sakata 2014) and zebra finches (Glaze & Troyer 2013), our measure also corrects for population-level consistency for each transition type. Some previous studies (Glaze & Troyer 2013; James & Sakata 2014, 2015, 2019; Ota & Soma 2014) assessed the effects of age on song using longitudinal data from the same individuals at different ages. In our study, each bird was recorded only once, so we cannot study changes over time within birds. Thus, variability in temporal features among birds might mask changes with age even if those changes did exist. In willow warblers (P*hylloscopus trochilus*), longitudinal and cross-sectional analysis of age-related changes in song gave different results, with changes in element rate not detected using cross-sectional analysis (Gil et al. 2001). Our dataset included few birds less than one year of age, which were the focus of some studies examining Java sparrows (Ota & Soma 2014) and zebra finches (Glaze & Troyer 2013), which may have resulted in the discrepancy between our findings and previous studies. However, this does not explain differences between our results and those from studies examining older birds (e.g., James & Sakata, 2015).

Social inheritance has the potential to influence song evolution and divergence in this species, as well as conservation efforts. The effect sizes in our models indicate that, whilst learning of traits is quite faithful, it is not completely accurate and error is likely to be introduced in each generation. Build-up of copying errors in different populations may result in divergence of song-timing characteristics among captive populations and between wild and captive populations. If differences in temporal features are salient and influence mate choice, as in other species (Nolan & Hill 2004; Dunning et al. 2020), these differences could influence the success of conservation programmes by influencing mating patterns (Lewis et al. 2021b), as in other species. Little is known about the features which influence mate choice in Java sparrows, so it is not possible to predict the effects that sexual selection may have on temporal song features. Even if not specifically selected during mate choice, selection for other features, such as note repertoire or number of notes, may influence temporal song features through associated changes in inter-note intervals, as these traits are linked in this and other species (e.g., Bengalese finch (Soma et al. 2006a)).

Overall, our findings suggest that social learning is the main mode of inheritance for many temporal song features in Java sparrows. However, we found evidence that song duration, is, at most, weakly socially learned, and partly constrained by a correlation between the number of notes and tempo. This suggests it is difficult for birds to increase their song duration, highlighting its potential as an honest indicator of mate quality. As such, examining the role of song duration in mate choice for this species could prove to be informative in determining how this potential honest indicator is perceived by females. Contrary to other species, we found no evidence to support an effect of genetic inheritance or the developmental environment, and little evidence to support the effect of age on most of the features examined. Based on this and studies in other species, it is likely that learning of temporal features interacts with vocal learning in the development of the adult song phenotype, which could be further examined through experimental studies.

4.7 Author contributions

RL, AK, and TG conceptualized the study. The original dataset was collated by RL and MS. RL, MS, SdK, and TG contributed to data extraction. RL, AK, and TG conducted data analysis. The initial draft was written by RL, with all authors were involved in reviewing and editing the manuscript. TG provided supervision throughout the project.

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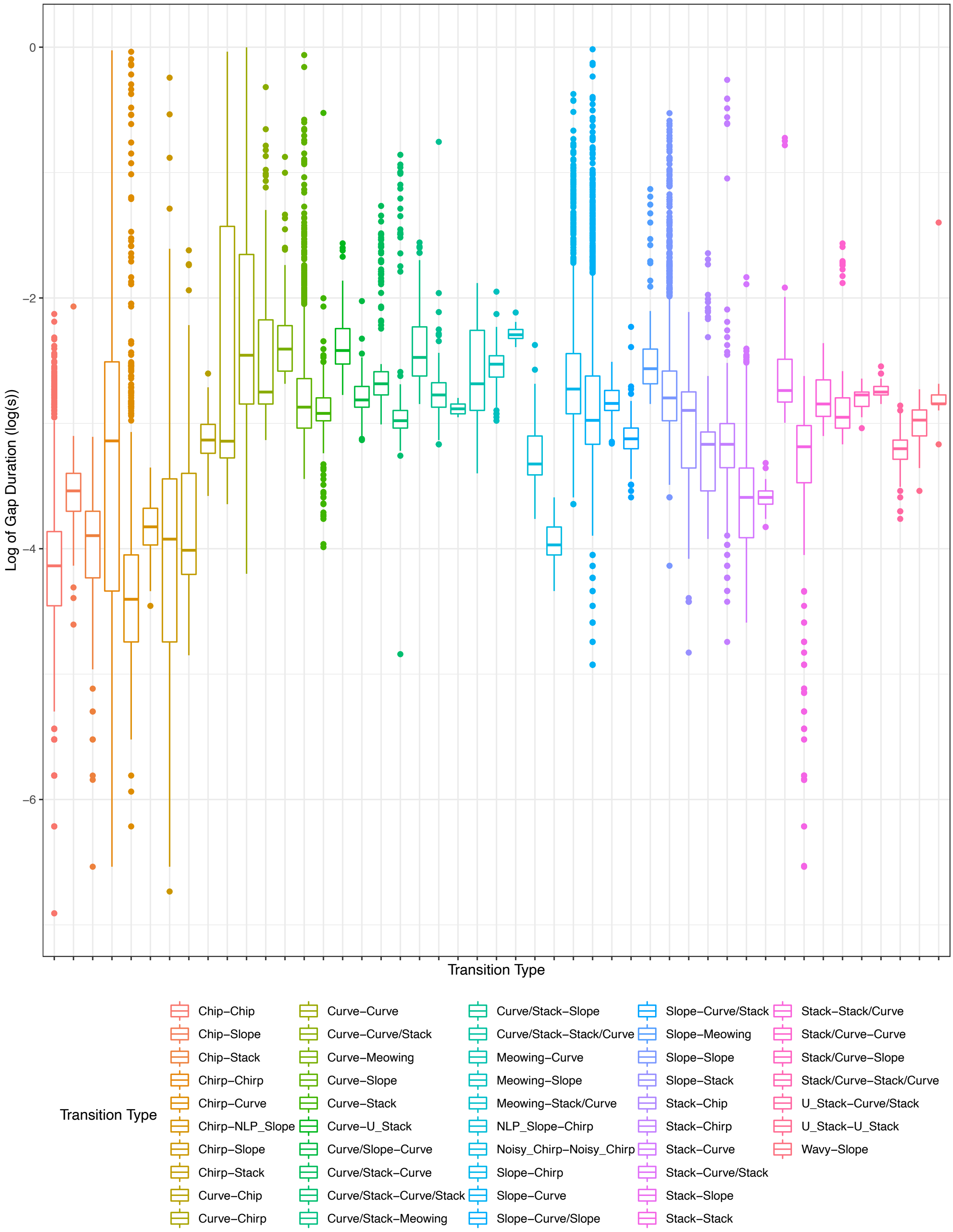
4.10 Supplementary Information

Supplementary Information 4.1: Lengths of transition types sung by Java sparrows in the study population. Transition types with more than 10 instances across the population are included. Note names associated with transition types reflect the categories described in Lewis et al. (2021a).



Chapter 5: Generalization of familiar song preference in Java sparrows

Rebecca N Lewis, Hiroharu Makioka, R Tucker Gilman, Masayo Soma

5.1 Chapter Summary

Many wild bird populations exhibit population-specific dialects, were populations have acoustically distinct vocalizations (Catchpole & Slater 2008). As discussed in Chapter 2, these vocal dialects can influence female mate choice, with potential knock-on effects for conservation programmes.

In Chapters 3 and 4, I established that social learning plays a crucial role in male song development in the Java sparrow, with males learning components of nearly all song features examined. Therefore, it is possible that this could contribute to the build-up of differences between populations of Java sparrows. To understand the possible implications of populations differences for Java sparrow conservation, we need to understand how such differences could influence female mate preference. As with song learning in males, female preference is learned early in life in a range of estrildid finch species (Clayton 1988; Fujii et al. 2021) and persists throughout adulthood (Fujii et al. 2021).

Many studies report that females prefer familiar songs. On a broad scale, this can include the preference for familiar dialects, which is discussed in Chapter 2. However, at a finer scale, specific songs within a population may be preferred. Preference for father's song has been reported in other estrildid finch species (Clayton 1988; Riebel 2000; Fujii et al. 2021; Fujii & Okanoya 2022). In this case, in addition to broad scale preferences, females may show preference for songs similar to those of their father even within their own population. Understanding preference for familiar songs at a fine scale is, therefore, an important precursor for predicting how birds may respond to familiarity across larger, population-level, scales.

In this chapter, I present a study examining female preference for familiar songs in Java sparrows. Females were presented with pairs of familiar vs. unfamiliar song stimuli and their behavioural responses were measured. A previous study in Java sparrows found no effect of song-related traits on association with live males, albeit with a small sample size. However, specific preferences for familiar songs have not yet been assessed. Therefore, this study is a useful first step in understanding how female preferences may shape ex situ breeding programmes and conservation efforts.

5.2 Abstract

Songs play an important role in mate choice for a range of bird species, and many birds show a preference for familiar, rather than unfamiliar, songs. These preferences could emerge due to sexual imprinting. However, very strong preferences for the parent's phenotype may be maladaptive and lead to inbreeding. It is, therefore, important to understand birds' ability to generalize stimuli used in mate choice to understand how mate preference may influence population dynamics. We examined female preference for familiar over unfamiliar songs in Java sparrows, where familiar songs were those of their social father or songs similar to their social father's song. We used song playback to present pairs of familiar and unfamiliar stimuli to females. We counted behavioural responses to determine preference, using a range of behaviours that are likely to indicate song preference in this species. Overall, we found that Java sparrow females preferred familiar over unfamiliar songs, suggesting that they were able to generalize preferences for their social father's song to other, similar songs. Even though birds are able to generalize their preferences, preference for familiar over unfamiliar songs could still contribute to assortative mating within populations. This may have a negative effect on population management and conservation breeding programmes in this species. Further investigation in natural conditions is necessary to fully understand the effects that preference for familiar songs could have on mating and reproductive success.

5.3 Introduction

Songs play an important role in mate choice for a range of bird species. Many female birds show preferences for specific song features, such as complexity (Vyas et al. 2009) and temporal characteristics (e.g., long, fast songs (Nolan & Hill 2004)), which may be related to male quality (Gil & Gahr 2002). Familiarity of songs may also influence female preference (Clayton 1990; Riebel 2000; Hernandez et al. 2009). For example, in species with macrogeographic population dialects, females tend to show preferences for their local (familiar) dialect over foreign (unfamiliar) dialects (Baker 1983; Macdougall-Shackleton et al. 2001; Searcy et al. 2002; Hernandez et al. 2009).

One mechanism by which preference for familiar songs could emerge is sexual imprinting, which is common in birds (ten Cate & Vos 1999). In this case, the parental phenotype is used as a model for later sexual preference by the offspring (Irwin & Price 1999; ten Cate et al. 2006). Learned preferences acquired through sexual imprinting may help individuals to identify conspecific mates later in life (Irwin & Price 1999). In zebra finches, cross-fostering experiments showed that early learned preferences for the parent's subspecies persisted later in life (Clayton 1990). Sexual imprinting can also be considered a type of generalized mate choice copying for the same-sex parent's mating decision (Schielzeth et al. 2008), which may be beneficial, as the opposite-sex parent is an example of a successful mate. Bateson (1978) suggested that sexual imprinting allowed for a balance between inbreeding and outbreeding where individuals choose a mate with a phenotype slightly different to that of their parent. In this way, very strong preference for parent's phenotype, with little generalization, may be maladaptive and result in inbreeding. As such, understanding birds' ability to generalize stimuli used in a mate choice context is important for understanding how preferences could shape population dynamics.

In estrildid finches, female preference for father's song has been reported in a range of species (e.g., Bengalese finches (Kato et al. 2010; Fujii et al. 2021; Fujii & Okanoya 2022), zebra finches (Riebel 2000)). These song preferences are formed early in life (Riebel 2003) and are learned; females removed from their father early in life may not develop preference for father's song (Clayton 1988) and females show preference for non-father tutor's songs (Riebel 2000), which indicates of some form of imprinting. Similarly, birds that are not exposed to species typical songs early in development show atypical preferences in adulthood. For example, female zebra finches which are denied access to a tutor do not show preferences based on song quality as adults (Lauay et al. 2004).

There is also evidence that females are able to generalize their learned preferences to other, similar songs. On a broad scale, this is demonstrated through preferences based on subspecies or macrogeographic population dialects, where females show preferences for dialect or subspecies songs that they were exposed to early in life, regardless of their origin (Clayton 1990; Hernandez et al. 2009). However, at a finer scale, specific songs within the population may be preferred, as is the case with father's song. Females may, therefore, prefer songs within their population that more closely resemble the songs of their father. Evidence for generalization at this scale has been well studied in zebra finches, but remains limited in other species. Early studies in zebra finches found that females' preference for father's song was less pronounced with compared to a more similar song (Miller 1979; Clayton 1988), suggesting that birds found it harder to discriminate when songs were more similar. However, females showed no preference for the songs of unfamiliar brothers over unfamiliar songs (Riebel & Smallegange 2003), even though brothers' songs were more similar to their father's song than unfamiliar songs. More recently, it has been found that adult zebra finches mate assortatively based on family-specific 'cryptic song dialects' identified using machine learning (Wang et al. 2022), suggesting preference for familiar songs may be based on small, subtle cues and has the potential to influence pair formation and breeding. Examining within-population preferences for familiarity in other species may help to further our understanding of birds' ability to generalize song preference.

We examined preference for familiar songs in Java sparrows, a member of the estrildid family. Males learn to produce a single song during a critical period, which is used solely in courtship (Ota

& Soma 2014). Courtship in this species also involves co-ordinated duet dancing (Soma & Iwama 2017). Whilst factors affecting mate choice in other estrildid finches, such as the zebra finch and Bengalese finch have been examined in depth, little is known about the factors affecting song preference and mate choice in this species. A study examining preference with live males found no effect of song-related traits on the time females spent near males (Hasegawa et al. 2011). To our knowledge, specific preference for song traits has not been assessed. We used song playback trials to examine female preference, which allows us to test song preference independently of other male traits (Fujii et al. 2022). We measured females' preference for their father's songs, and songs similar to their father's song, compared to unfamiliar songs to determine if birds were able to generalize their preference. We expect that females will prefer their father's song, and that this preference will extend to the songs of other males that match the father's song type.

5.4 Methods

5.4.1 Subjects

Subjects were 17 cross-fostered adult female Java sparrows. During rearing, birds were housed in family groups in separate cages, which were visually but not audibly isolated from other birds. Females belonged to nine different social family lines. Sons learn their songs from their social father (Lewis et al. 2021a). As such, each family line has a distinctive song type, which relates to their social pedigree i.e., birds which are socially related share a song type, and these song types can also be visually confirmed. We considered the social father's song, and songs from birds in the same social line as the social father (which share the same song type), to be familiar. Henceforth, we will use 'father' when referring to the social father, and 'line' when referring to birds that come from the social father's family line. See Appendix 1 for further details on subjects and housing.

5.4.2 Stimuli

Three types of stimuli were created for use in the experimental trials. The 'Father' stimulus was made up of songs recorded from the female's father. 'Line' stimuli were made up of songs from a bird that had the same song type as the female's father, i.e., from the same social family. Where fathers had the opportunity to learn from multiple tutors, visual inspection was used to confirm the father's social line. In all but one case, the father's (and therefore the female's) social line was that of the father's main tutor (father's social father). However, in one case, the female's father closely resembled his subtutor's song (a tutor that was not the social father), and so was assigned to the subtutor's social line. 'Unfamiliar' stimuli were made up of songs from a bird that had a different song type to the father. Each female was assigned two unfamiliar stimuli (Figure 5.1; see also section 5.4.3 below). For each stimulus, three songs from a single male were selected from those available in archival recordings. Archival recordings had 44.1 kHz sampling rate and 16-bit resolution and consisted of an individual bird singing alone. Recordings were taken recorded using a digital sound recorder (Marantz PMD 661, Zoom Q3HD, TASCAM DR-100 MKIII). Recordings were high-pass filtered at 375 Hz in Raven Pro (1.6.3) to remove background noise. Recordings from four birds from one social line in the dataset had been pre-processed with a high pass filter of 1 kHz. We do not expect this to have an effect on preference tests; a high pass filter of 1 kHz is unlikely to have removed any important song features, and the closely related zebra finch (as well as other song birds) are reported to have a narrow hearing range with highest sensitivity between 1-6 kHz (Hashino & Okanoya 1989). The three selected songs for each bird were repeated at a constant rate (one song every 10s) for two minutes, alternating between songs. Many of the archival songs had been recorded with recorders using stop start direction (so only significant noise events were recorded), or, had been segmented into individual songs prior to storage. As such, we did not have information on the natural song rates of individuals in our dataset, and there is little published information on song rates in this species. Since song rate can influence preference (Forstmeier 2004; Nolan & Hill 2004), we chose to control for song rate during playback. The chosen rate may differ to natural song rates, and natural song rates are likely to differ between individuals and social contexts, as in other species (e.g., zebra finch (Dunn & Zann 1996; Naguib et al. 2008; Jesse & Riebel 2012)). Due to our methods of stimulus construction, the total time occupied by songs differed between stimuli, as individuals had differing song lengths. However, familiar and unfamiliar song stimuli differed for each individual female, and, as such, song density is unlikely to have systematically differed with familiarity. A full stimulus contained 12 songs in the pattern ABCABCABCABC. For all stimuli, amplitude was equalized by matching peak amplitude between stimuli, as song amplitude may influence preference (Ritschard et al. 2010).

Similarity between Father and Line stimuli was calculated based on a) the number of the father's notes appearing in the Line stimulus, and b) the overall number of shared notes between Father and Line stimuli. Notes were classified within social lineages based on structure in the spectrogram. At the level of classification used, it is not possible to compare notes between different social lines. This requires broader categorization of notes (as in Lewis et al., 2021a), which may not reflect finer differences in note structure used by birds. All classifications were made by a single observer. However, these classifications were repeatable between observers. In a sample of 146 songs (containing 4915 notes), a second observer was able to correctly classify 95.2% of notes at this using a provided list of exemplars (NB this dataset includes some songs from birds not represented in this study, and all birds in this study do not appear in the dataset). Across familiar pairs, an average of 91.4% of father's notes were represented in the 'Line' stimulus (interquartile range 81.8-100%; range 57.1-100%) and 72.7% of notes were shared between 'Father' and 'Line' stimuli (interquartile range 58.6-93.8%; range 26.8-100). Figure 5.1 shows an example of a set stimulus songs used for a single female (Father, Line, Unfamiliar 1, Unfamiliar 2).

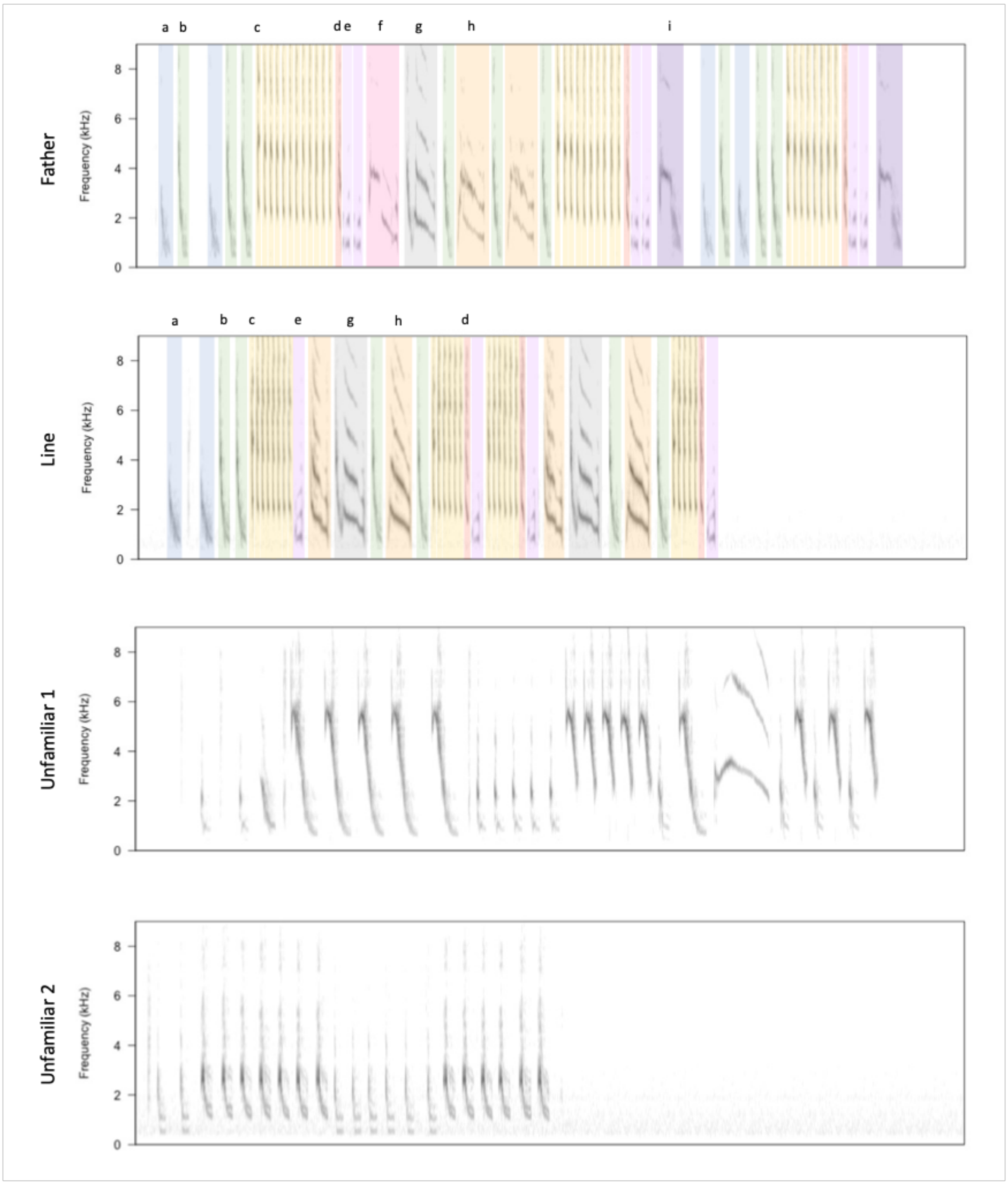


Figure 5.1: Example of stimuli presented to a single female. The 'Father' and 'Line' stimuli have been marked to show equivalent note types. Due to the variable nature of songs between different social lines, it is not possible to extend this level of classification to songs of other family lines, so 'Unfamiliar' stimuli have not been marked. Spectrograms were created using Seewave (Sueur et al. 2008) (sample rate = 44.1kHz, window length = 512, overlap = 90%).

5.4.3 Experimental trials

There were two types of trial during the experiment, Father vs. Unfamiliar and Line vs. Unfamiliar. To avoid habituation to the unfamiliar song, the song of a different unfamiliar male was used in each trial type (Figure 5.1). Line and unfamiliar birds were selected at random for each female. Where possible, clutch mates were avoided, but in two cases alternative familiar birds were not available. For two birds, recordings of the father's song were not available, so two Line vs. Unfamiliar stimulus pairs were presented, with a different familiar and unfamiliar bird in each pair.

Females completed four trials during the experiment. Each trial type was completed twice, with the order of presentation reversed in each repeat (Figure 5.2). The order of trials was randomized among birds. One of the 17 birds was removed from the study after completing a single trial. Within females, each trial took place on a different day. In total, 65 trials were recorded for analysis. Prior to each trial, females in individual cages were placed in a soundproof chamber and allowed to habituate. At the start of each trial, females completed a short habituation period (5 minutes). After this, females were presented with four stimuli (2 minutes), alternating between familiar and unfamiliar, with a 30 second gap between each stimulus presentation (Figure 5.2). Each trial lasted 10 minutes.

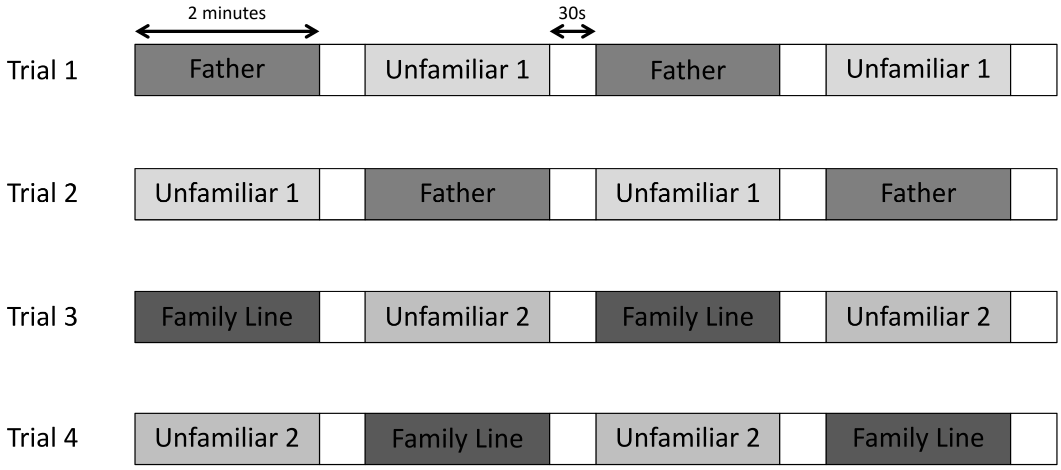


Figure 5.2: Example experimental set up for a female completing the experimental procedure.

5.4.4 Behavioural data

Information on behavioural responses to stimuli were extracted from video footage taken during trials. We examined four behaviours, which are performed in a range of social situations by Java sparrows: calls, hops, bill wiping and fluffing. These behaviours were chosen to cover a wide range of social responses, including behaviours used in courtship displays, as these may indicate mate preference. In Bengalese finches, calls were a reliable indicator of preference, showing similar patterns to copulation solicitation displays (Dunning et al. 2014). In Java sparrows, both hopping and bill wiping are associated with courtship dancing (Soma & Iwama 2017), so may indicate mate preference. In zebra finches, fluffing behaviour is associated with mate preference and is performed in response to complex, attractive songs (Vyas et al. 2009). Juveniles experiencing more fluffing behaviour in response to songs also developed more accurate songs (Carouso-Peck & Goldstein 2019; Carouso-Peck et al. 2020). Instances of each behaviour were counted for each stimulus presentation within the trial (i.e., each 2-minute block of song playback). Behaviours occurring in the 30s gap between stimulus sets were not counted, as it is harder to interpret the meaning behind behaviours performed during this time period.

5.4.5 Data analysis

All analyses were conducted in R (ver. 4.1.2). We conducted separate tests for each of calls, hops, bill wiping and fluffing. Due to the number of non-responses from females, it was possible that our data were zero-inflated. Models were initially constructed without zero-inflation and checked using the performance package in R (Ludecke et al. 2021). Where likely zero-inflation was detected, we re-ran the models including a zero-inflation term. As such, bill wiping and fluffing were analysed with a generalized linear mixed effect model (with Poisson error distribution) using the lmerTest package (Kuznetsovs et al. 2017), and calls and hops were analysed using a zero-inflated Poisson models using the glmmTMB package (Brooks et al. 2017). Across models, the behaviour of interest was used as the response variable. We included the type of stimulus (familiar or unfamiliar), the type of trial (Father vs. Unfamiliar or Line vs. Unfamiliar), the order of the stimulus within the trial (played 1st, 2nd, 3rd or 4th), and the order of the trial within the experimental period (1st trial, 2nd trial, 3rd trial or 4th trial) as fixed effects in the model. The order of the stimulus was included to account for habituation within the 10-minute trial. The order of the trial was included to account for habituation over the course of the overall experiment. We also included the interaction between the type of stimulus and the type of trial to determine if the difference in response to familiar songs was different in Father vs. Unfamiliar or Line vs. Unfamiliar trials. We included the female ID, the ID of the male’s song used and female ID x male ID as random effects in the models. Where used, the zero-inflation formula included an intercept and a random effect of the individual female. This accounted for the fact that females may not respond during the presentation of a stimulus during the trial, and that the likelihood of not responding differed among females.

5.4.6 Ethical approval

This study was approved by The University of Manchester Animal Welfare and Ethics in Research Board and Hokkaido University.

5.5 Results

Overall, we found evidence that Java sparrow females preferred familiar (Father or Line) over unfamiliar songs. Significantly less hops and fluffs were performed during playback of unfamiliar songs (Table 5.1, Figure 5.3). A similar, marginally significant pattern was observed for the number of calls (Table 5.1, Figure 5.3). The number of bill wipes followed the same pattern, but this did not reach marginal significance (Table 5.1, Figure 5.3).

Table 5.1: Results of mixed effect models for behavioural responses. Estimates show comparison of unfamiliar to familiar songs and Line vs. Unfamiliar (L vs. U) to Father vs. Unfamiliar test types.

	Estimate	p-value	Direction of effect
Number of Calls			
Unfamiliar	-0.92	**0.066**	Familiar songs preferred
Test Type (L vs. U)	0.23	0.632	N.S.
Test Order	0.18	**<0.001**	Acceleration effect
Stimulus Order	-0.22	**<0.001**	Habituation effect
Unfamiliar : Test Type (L vs. U)	0.45	0.507	N.S.
Number of Hops			
Unfamiliar	-1.32	**<0.001**	Familiar songs preferred
Test Type (L vs. U)	-0.58	0.108	N.S.
Test Order	-0.10	**<0.001**	Habituation effect
Stimulus Order	-0.09	**<0.001**	Habituation effect
Unfamiliar : Test Type (L vs. U)	0.97	**0.059**	Smaller difference in response in Line vs. Unfamiliar trials
Number of Bill Wipes			
Unfamiliar	-0.55	0.385	N.S.
Test Type (L vs. U)	-0.76	0.210	N.S.
Test Order	0.36	**<0.001**	Acceleration effect
Stimulus Order	0.09	0.312	N.S.
Unfamiliar : Test Type (L vs. U)	1.05	0.216	N.S.
Number of Fluffs			
Unfamiliar	-1.09	**0.014**	Familiar songs preferred
Test Type (L vs. U)	-0.67	**0.072**	Less response in Line vs. Unfamiliar trials
Test Order	0.08	0.518	N.S.
Stimulus Order	-0.27	**0.019**	Habituation effect
Unfamiliar : Test Type (L vs. U)	1.16	**0.049**	Smaller difference in response in Line vs. Unfamiliar trials

We did not find evidence for an effect of test type; females did not respond differently in Father vs. Unfamiliar trials compared to Line vs. Unfamiliar trials. The effect of test type was not significant for calling, hopping, or bill-wiping behaviours (Table 5.1). For fluffing behaviours, there was a marginally significant effect of test type, with birds responding less in Line vs. Unfamiliar compared to Father vs. Unfamiliar trials (Table 5.1).

However, there was some evidence for an interaction between stimulus familiarity and the test type. For fluffing behaviour, there was a significant interaction between familiarity and test type (Table 5.1); the preference for the familiar stimulus was less pronounced in Line vs. Unfamiliar compared to Father vs. Unfamiliar trials. A similar pattern of marginal significance was found for the number of hops (Table 5.1). Model estimates for the number of calls and the number of bill wipes were in the same direction but did not reach marginal significance (Table 5.1).

Generally, birds habituated to stimulus presentation within the 10-minute trial, responding less to stimuli presented later in the trial. We found a significant decrease in in calling, hopping, and fluffing over the trial (Table 5.1). However, there was no evidence for habituation when considering bill-wiping behaviour (Table 5.1).

Our results for changes in behaviour over the course of the whole experiment were less clear-cut. Whilst there was an apparent decrease in hopping behaviour across experimental trials, this was not the case for other behaviours (Table 5.1). We found no significant differences in fluffing behaviour across the course of the experiment (Table 5.1). However, for calling and bill-wiping behaviour, we found evidence for an acceleration effect; birds showed greater responses in later trials (Table 5.1). This suggests a change in response type to less energetic behaviours over the course of the experiment.

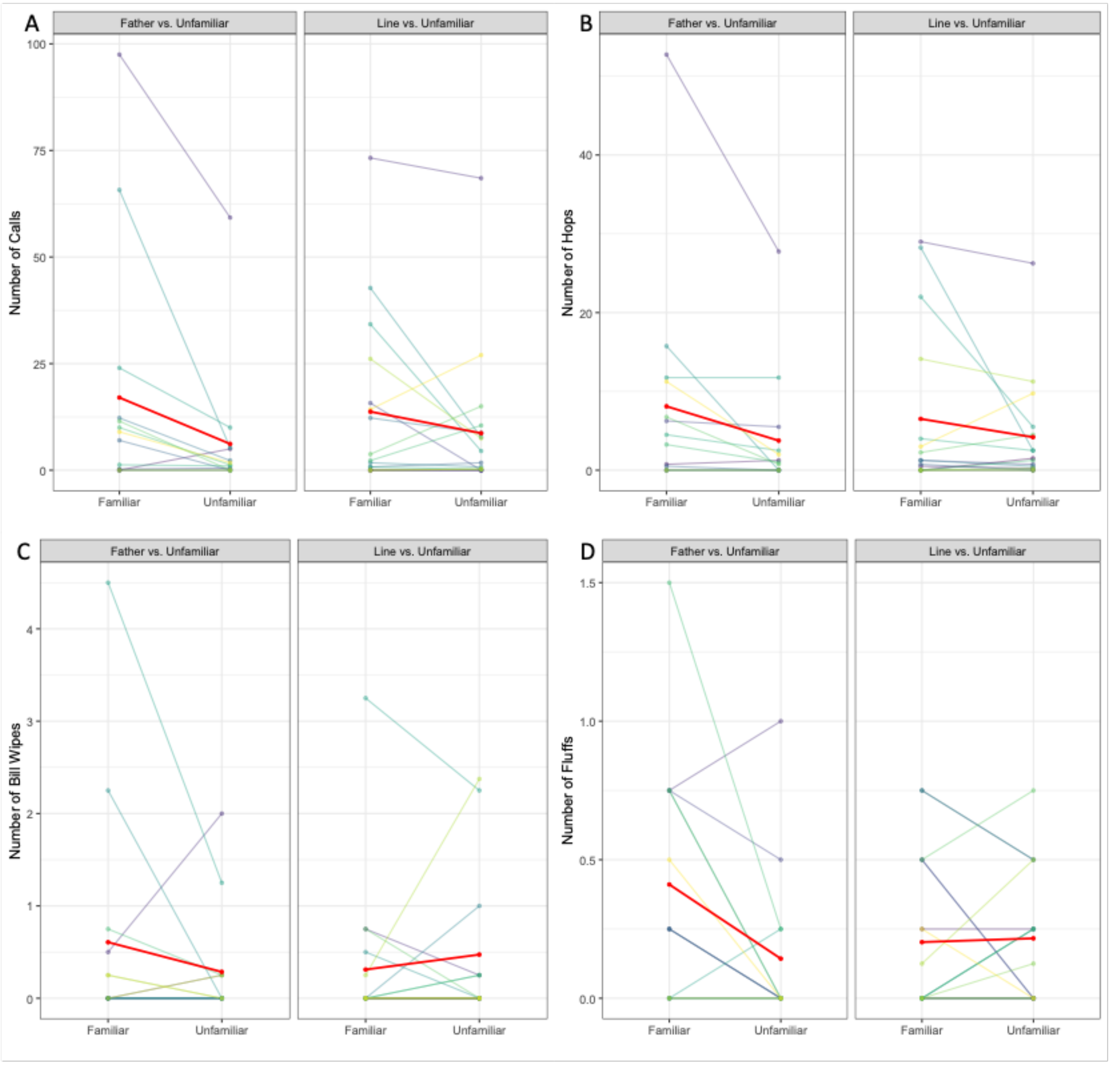


Figure 5.3: Behavioural responses from females to familiar and unfamiliar songs in Father vs. Unfamiliar and Line vs. Unfamiliar trials A) calls B) hops C) bill wiping D) fluffing. Transparent lines represent individual females' responses. Bold red lines indicate the average response across individuals within the trial type.

5.6 Discussion

Java sparrow females preferred familiar over unfamiliar songs, where familiar songs were produced by the social father ('Father') or other birds with the same song type as the social father ('Line').

Preference for father's song is found in other, closely-related species (Riebel 2000; Kato et al. 2010; Fujii et al. 2021; Fujii & Okanoya 2022). Female Bengalese finches show preference for their father's song over unfamiliar songs in operant tasks (Kato et al. 2010) and based on behavioural responses (Fujii et al. 2021; Fujii & Okanoya 2022), including copulation solicitation displays (Fujii & Okanoya 2022). Similarly, zebra finches show preferences for their tutor's song in an operant task (Riebel 2000). Java sparrows were also able to generalize preference for the father's song to other, similar songs. A wide range of song features, including song and note structure, are socially inherited in this species (Lewis et al. 2021a). As such, song type and song similarity likely indicate that birds are (socially) related. Evidence for generalization from other estrildid finch species is mixed (Table 5.2). In Bengalese finches, although the direction of response indicated preference for unfamiliar songs that were more similar to the father's song, the tests did not reach statistical significance (Fujii & Okanoya 2022). A study in zebra finches found that birds that had previously shown a preference for father's song over unfamiliar song did not show a similar preference for an unfamiliar brother's song (which was similar to the father's song) (Riebel & Smallegange 2003). Unfamiliar brothers shared ~53% of elements with their father. In Java sparrows, song learning of elements and their structure is very faithful (Soma 2011; Lewis et al. 2021a). In our study, a high proportion of the father's note types were present in the similar song (91.4%) and the percentage of shared elements between father's and similar songs was also relatively large (72.3%). As such, we may have been more able to detect generalized preferences due to higher song similarity. In line with this, Wei et al., (2022), found that zebra finches exhibited stronger preferences for songs sharing more elements with father's song. Many previous studies have used operant tasks (e.g., (Riebel & Smallegange 2003; Wei et al. 2022); Table 5.2) or examined few behavioural responses, such as copulation solicitation displays (Fujii and Okanoya, 2022; Table 5.2) or approach (Miller; Clayton 1988; Table 5.2). Our study examines the combined evidence from multiple behavioural parameters in a naturalistic context and without interventions such as hormone implants, allowing us to more readily assess the social role of songs in this species.

We did not find strong evidence to suggest that father's song was more strongly preferred than familiar songs from other birds. There was some evidence for an interaction between test type and stimulus familiarity in two of the behaviours; the difference in response between familiar and unfamiliar stimuli was larger in Father vs. Unfamiliar trials compared with Line vs. Unfamiliar trials. These findings are not strong enough to draw definitive conclusions about differences in preference strength. To limit the number of trials undertaken by females we did not directly compare preference for father and line songs, so it is not possible to determine if father's song was more strongly preferred. However, this is a potentially valuable avenue for future studies.

Responses to father's song could simply reflect interest in a known stimulus. However, similar songs were not generally known to birds (except for two cases where the only non-father familiar song available was from a nestmate), so familiarity with the specific stimulus does not fully explain the preferences observed in this study. The behaviours we examined are likely to indicate song *preference*, rather than a simple response. Calls are associated with female preference in Bengalese finches; calls and copulations solicitation displays were correlated in determining song preferences (Dunning et al. 2014). In zebra finches, fluffing behaviours were performed more frequently in response to complex songs in both untreated and hormone-treated females (Vyas et al. 2009). Finally, both hops and bill wipes are used by Java sparrows in courtship display dancing (Soma & Iwama 2017). Therefore, these behaviours are likely to correlate with mate preference.

Table 5.2: Summary of existing literature on preference generalization in estrildid finches

Species	**Comparisons and method**	**Song similarity**	**Sample size**	**Results**	**Reference**
Zebra finch	Father vs. similar and Father vs. dissimilar; behavioural indicators (approach)	Not directly stated, similar songs obtained from uncles or siblings	31 (F vs. DS), 21 (F vs. S), all females	Significant preference for father over dissimilar, with lower latency to approach and higher duration of time spent near stimulus, no significant preference for father over similar songs, with no significant difference in latency, but higher duration	Miller 1979
Zebra finch	Father vs, Similar, Father vs. Dissimilar, Tutor vs. Similar, Tutor vs. Dissimilar; behavioural indicators (calls, approach)	Similar songs shared at least 75% of elements with father/tutor	17 raised with father to 35 days (Group B), 17 raised with father to 35 days, with a tutor from 35 to 70 days, and a second tutor between 120 and 180 days (Group C), males and females	Group B birds had a stronger preference for the father in tests with dissimilar songs. Group C had equal, strong preference for father in both test types, with a similar pattern for tutor 2, but stronger preference for tutor 1 in tests with dissimilar songs	Clayton 1988
Zebra finch	Unfamiliar brothers; operant task	53 +/- 10% shared elements	18 (8M, 10F)	No significant preference for song of unfamiliar brother over unfamiliar songs	Riebel and Smallegange 2003
Zebra finch	Assortative mating based on rearing history; mate preference	Not specifically stated, examined categorical (same rearing environment) and	4 population groups of cross-fostered individuals, 3 with 40:40	Birds in Generation 2 showed positive assortative mating with individuals from population of rearing, suggesting learnt preference for a cultural trait. Associations were related to similarity of male's	Wang et al. 2022

		continuous (song similarity quantified by Sound Analysis Pro and a machine learning algorithm)	male:female and one with 21:31	song to songs of the individual with whom females grew up (peers). Assortative mating in Generation 3 was related to culture of foster grandparents in Generation 1	
Zebra finch	Similarity gradient from father's song to unfamiliar; operant task	Similar songs shared 2/3 elements with father's song and 1/3 elements with father's song	23 females (16 reached inclusion criteria)	Father's song visited more than other categories, songs sharing 2/3 of elements were preferred over those sharing 1/3 and no elements. Visit duration was longest for father's song but more for songs sharing 2/3 and 1/3 elements compared to unfamiliar songs	Wei et al 2022
Bengalese finch	Father vs. four unfamiliar; behavioural indicators (copulation solicitation displays)	Similarity to father's song calculated using Sound Analysis Pro or based on tempo similarity	10 females	Father's song preferred over unfamiliar for number of CSDs. Non-significant when considering duration of CSDs. Trend towards greater response to unfamiliar songs based on similarity to father	Fujii et al., 2022
Java sparrow	Father vs, Unfamiliar, Song Similar to Father vs. Unfamiliar; behavioural response (multiple behaviours)	91.4% of father's elements represented in similar song, 72.7% elements shared between father and similar	17 females	Birds showed preference for familiar (Father or Song Similar to Father) over unfamiliar songs; little evidence for differences in response between the two test types	This study

Whilst preference for familiar songs in a mate choice context can assist correct choosing of subspecies etc., strong preference for familiar songs in a mate choice context could contribute to inbreeding by promoting assortative mating based on population (genetic) background (Soma 2011). In zebra finches, females preferentially paired with males whose song matched their population of rearing and that more closely resembled songs of their adolescent peers (Wang 2022). As Java sparrows show familiar song preferences, as do zebra finches, it is possible that similar patterns of mate choice could occur. Assortative mating based on song familiarity could negatively affect population management strategies that bring birds from multiple populations together, for example, during conservation breeding programmes (Lewis et al. 2021b). Even if females do not show a preference when pairing, investment in breeding may be reduced with non-preferred males. In Bengalese finches, females produced heavier eggs and showed a trend towards male-biased broods when their mate had a longer song duration (Soma & Okanoya 2013). As such, pronounced preferences for familiar songs might contribute to the formation of a pre-mating reproductive barrier. Mate choice in natural situations may not always reflect preference in controlled settings. Other factors, such as additional song parameters (Nolan & Hill 2004; Dunning et al. 2020) and male traits (Hasegawa et al. 2011), may influence pairing. In the present study, females varied in their strength of response and preference. As such, individual differences may also contribute to mate choice in real-world situations. Our results add to the growing evidence from zebra finches and Bengalese finches that birds can generalize their learned preferences acquired early in life to other, similar stimuli; Java sparrows showed preference for both their father’s and songs similar to those of their father compared to unfamiliar songs. Further investigation in natural conditions is necessary to understand the implications of preference for familiar songs as a pre-mating barrier and its potential to contribute to reproductive isolation between populations.

5.7 Author Contributions

RL and MS conceptualized the study and were responsible for the experimental design. Data collection and extraction of behavioural data from videos was carried out by HM. Data analysis was conducted by RL, MS, and TG. The initial draft of the manuscript was written by RL, with RL, MS, and TG contributing to reviewing and editing. MS and TG provided supervision throughout the project.

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Chapter 6: Does ex situ breeding influence size and sexual dimorphism in an estrildid finch?

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6.1 Chapter summary

In previous chapters, I have discussed some of the potential influences of ex situ breeding on vocal behaviour and its associated responses. However, ex situ breeding may also result in changes to other phenotypic and genetic characteristics. As well as these changes being important in their own right, changes in correlated traits could influence vocal change and divergence during ex situ breeding programmes. Morphological differences in captive environments have been reported across a range of taxa (Kraaijeveld-Smit et al. 2006; Bernatchez et al. 2008; Kamaluddin et al. 2019; Siciliano-Martina et al. 2021b, 2021a; Stojanovic et al. 2021). Morphology is important for a number of behaviours crucial for survival and reproduction. As well as potential effects on diet and feeding ecology (Grant & Grant 1989; Felice et al. 2019), bird morphology can also play a role in vocal communication, with body size and beak shape influencing vocal production (Podos 2001; Derryberry et al. 2018; García & Tubaro 2018). As such, significant morphological change in ex situ breeding programmes could result in poor performance of captive individuals released as part of reintroductions and supportive breeding programmes, as well as promoting vocal divergence between populations. Furthermore, if morphological differences in captivity are associated with genetic change, supportive breeding programme could influence wild phenotypes, reducing the fitness of wild populations.

In this chapter, I examine morphological differences between wild and captive populations of Java sparrows. Captive birds were represented by two populations, birds from a zoological collection and avicultural birds from a laboratory population. Wild birds were represented by museum skins. I used a range of morphological measurements, including beak and skull measurements, tarsus length, tail length, and wing length. As well as determining overall morphological differences, I also examine the extent of sexual size dimorphism in the various populations, as levels of dimorphism may also be influenced by ex situ breeding. As Java sparrows are common in captive environments, they are a good candidate for assessing adaptation to captivity as there is substantial potential for population differences to develop.

6.2 Abstract

Captive populations are an integral part of conservation programmes. However, phenotypic changes in captivity may result in captive-born individuals having poor performance compared to wild conspecifics in situ. Morphological differences between wild and captive populations have been reported across a broad range of taxa, and, as such, differences in morphology may be an easy-to-measure indicator of captive adaptation. We examined morphology and sexual size dimorphism in three groups of Java sparrows, museum specimens (wild), zoo-bred, and avicultural. Among males, avicultural birds were larger than those from the museum population across measures, and larger than zoo birds for some measures. Zoo males were also larger than museum males for most measures. Patterns were less pronounced among females; avicultural females were larger than museum females for some measures, but zoo females did not differ significantly from avicultural or museum females. In addition, we found that both avicultural and zoo birds showed significant sexual size dimorphism across a number of measurements, but no sexual size dimorphism was apparent in museum birds. These results suggest that individuals from captive origins could be poorly adapted if used in reintroduction or supportive breeding programmes, and males may be more negatively affected than females. Differences in morphology could result from a plastic response to changed environments in captive conditions, inadvertent selection in captivity, sexual selection, or, some combination of these influences. The relative contributions of these mechanisms will affect the consequences of morphological differentiation during conservation, with higher contributions from selection leading to possible negative consequences for in situ populations.

6.3 Introduction

Captive populations of animals are an integral part of conservation programmes, and the release of captive individuals into the wild is an important conservation tool. As such, maintaining genetic diversity and reducing genetic change in captivity are key goals in conservation breeding programmes (Frankham et al. 2002; Frankham 2008; Williams & Hoffman 2009; Hvilsom et al. 2022). However, captive environments are necessarily different from wild environments and, as such, captive populations are subject to different conditions and selection pressures than those in the wild. This, in turn, can result in a series of interlinked phenotypic and genetic changes in captive populations (Bernatchez et al. 2008; Frankham 2008; Williams & Hoffman 2009; Chargé et al. 2014). As a result, captive-born individuals may have poor performance in wild environments compared to their wild conspecifics (Kraaijeveld-Smit et al. 2006; Milot et al. 2013). For example, whilst anti-predator responses in Mallorcan midwife toads (*Alytes muletensis*) were retained across a short number of generations, responses started to degenerate after 9-12 generations in captivity (Kraaijeveld-Smit et al. 2006). Release of poorly adapted captive animals may also have a negative effect on the wild population, including changes in behaviour and morphology, as well as genetic effects such as the loss of local adaptation (Lynch & O'Hely 2001; Ford 2002; Champagnon et al. 2012). Therefore, it is important to examine the potential for adaptation to captivity in species involved in conservation breeding programmes to identify and mitigate against any potential issues.

Morphological differences between wild and captive populations have been found across a broad range of taxa (mammals (e.g., Kamaluddin et al., 2019; Pelletier et al., 2021; Siciliano-Martina et al., 2021b, 2021a), birds (e.g., Remeš & Székely, 2010; Soma, 2005; Stojanovic et al., 2021), amphibians (e.g., Kraaijeveld-Smit et al., 2006), fish (e.g., Bernatchez et al., 2008; Pulcini et al., 2013)). These differences can be related to a range of factors, including responses to environmental conditions, such as altered or improved diet (Kamaluddin et al. 2019; Siciliano-Martina et al. 2021a), or due to inadvertent selection for morphology or other linked traits (Chargé et al. 2014). In some cases, selective breeding for certain traits, e.g., during domestication or aviculture, can also influence morphology in captive populations (Remeš & Székely 2010). Morphological measures can be taken quickly, easily, and at low cost in most species, making them a useful indicator to identify subtle changes during captive breeding. Additionally, morphological measures may be associated with other changes that are harder to quantify. For example, in Atlantic salmon (*Salmo salar*), captive and wild born individuals differed in morphology of the head and pectoral fins (Bernatchez et al. 2008). The groups were also genetically different, with captive born individuals having different allelic frequencies and reduced heterozygosity and allelic richness compared to wild born individuals. Whilst no specific link was made between genetic and morphological differences, these findings highlight the potential for combined genetic and morphological change in captive individuals.

In birds, morphology is important in a range of behaviours crucial for survival and reproduction, including diet and foraging (Grant & Grant 1989; Felice et al. 2019), and vocal communication (Derryberry et al. 2018; García & Tubaro 2018). Therefore, changes in morphology may influence survival post-release during reintroductions or supportive breeding. As well as being bred as part of conservation breeding programmes in zoos, birds are also frequently bred by aviculturists, with many endangered species represented. Birds in aviculture could be used to bolster conservation efforts and increase genetic diversity in captive breeding programmes, with birds moving from avicultural populations into zoo populations and vice versa. However, this may not be suitable if avicultural birds are significantly divergent from wild or other captive individuals, which could occur due to selective breeding for desired traits such as size and colour.

It is possible to examine the effects of captivity on morphology by comparing captive individuals to those of wild origin e.g., museum specimens. In this study, we examine the morphology of Java sparrows (*Lonchura oryzivora*), an endangered estrildid finch species (BirdLife International 2018). We compared individuals from three different origins: museum specimens, zoo-housed birds, and birds from aviculture. The prevalence of Java sparrows in captive collections, both zoological and avicultural, make it a good candidate for assessing adaptation to captivity and determining the feasibility of using avicultural birds to bolster conservation breeding programmes, as there is a high potential for population differences to develop.

6.4 Methods

6.4.1 Study species

Java sparrows are an estrildid finch native to Indonesia and found as an invasive species in many countries within South East Asia and beyond (Restall 1996; BirdLife International 2018). The species is frequently kept in zoological collections and is a popular bird in aviculture. Adult Java sparrows have monotypic plumage. Both males and females have predominantly grey plumage, with black heads, white cheeks, and pink beaks (Restall 1996). Juvenile birds have typically brown plumage with dark brown bills.

6.4.2 Populations

We examined birds from three different origins; wild birds from museum collections, captive bred birds from a zoological collection, and avicultural birds from a laboratory population.

6.4.2.1 Museum

Data from museum birds came from three separate collections (Natural History Museum at Tring, Liverpool World Museum, Museum für Naturkunde Berlin). Collection dates of museum specimens ranged from 1766 to 1989. Juvenile birds (identified by plumage as described above) were excluded from analyses, as we expected these to have significantly different morphology to adult birds. Birds that were likely to be of captive origin were also excluded from analyses. This included birds where captive status was included on specimen labels and birds that were from outside of the recorded range for Java sparrows (i.e., Europe). Locations of birds were checked against recorded ranges from a number of sources to determine suitability for inclusion (Restall 1996; BirdLife International 2018). We also removed birds with no location information from the dataset, as the origin of these birds was not possible to determine. A single white morph was excluded from analyses, as unusual colour morphs may exhibit different traits to wild-type individuals. Sexes were determined based on the museum catalogue/labels, and only birds of known sex were included in the analyses. In total, 58 birds met the above criteria (25 female, 33 male).

6.4.2.2 Zoological

Zoological birds were housed in a large, indoor, free-flight enclosure at Chester Zoo. Birds in this population were allowed to breed freely, with no external selection factors (e.g., assigned breeding partners). Birds were measured post mortem. Feather samples were taken and sent for genetic sexing (STAB VIDA Bird Sexing). Birds with evidence of juvenile plumage were not included in the analyses. In total, 77 adult birds were measured, of which 57 were successfully sexed and included in the study (19 female, 38 male)).

6.4.2.3 Avicultural

Avicultural birds were measured in a laboratory population housed at Hokkaido University, Japan. Birds in this population were originally sourced from avicultural breeders, with some birds being bred in the lab. Live birds were measured and returned to their usual environment following handling. All birds measured in this population had full adult plumage, so were classed as adults. No birds with juvenile plumage were present in the population at the time of measuring. Unusual colour morphs (white (n=5) and fawn (n=2)) were excluded from analyses. Selective breeding for colour morphs is likely to have exerted different artificial selection on these birds than wild type individuals, and unusual colour morphs are unlikely to be moved to zoological collections or included in supportive breeding programmes. Sexes for birds in the laboratory population are known and recorded, so no further sex determination was required. A total of 154 birds were measured and included in the dataset (72 female, 79 male).

6.4.3 Measurements

In total, seven beak and body measurements were taken for each bird; beak width (the widest part of the top of the beak), beak length (culmen, the tip of the beak to the point where the upper mandible meets the feathers), beak height (the base of the beak to the highest point of the upper beak), skull length (the back of the head to the tip of the beak), tarsus length (the inner bend of the tibiotarsal articulation to the base of the toes), natural wing length (from the bend of the wing to the tip of the longest primary flight feather, maintaining natural curvature), and tail length (base of the tail to the tip of the longest tail feather) (Figure 6.1). Wing lengths from museum specimens were not used in analyses, as wings were fixed during specimen preparation and measurements were therefore unlikely to correspond with the natural wing lengths taken for avicultural and zoological birds.

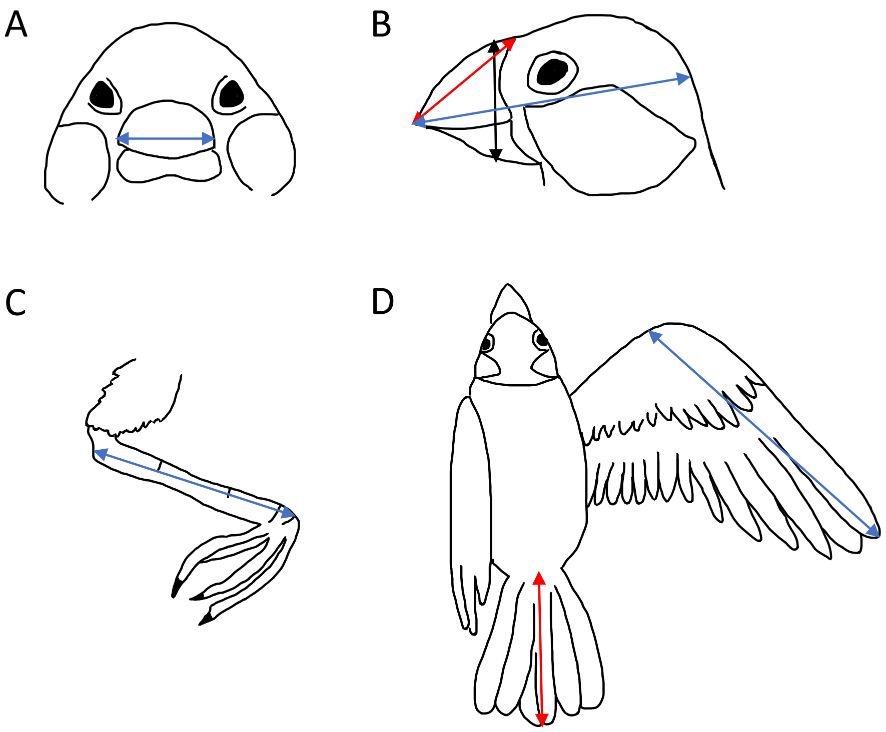

Figure 6.1: Measurements taken from Java sparrow. Panel A) Beak width (blue arrow) Panel B) Beak Length (red arrow), Beak height (black arrow), Skull length (blue arrow) Panel C) Tarsus length (blue arrow) Panel D) Tail length (red arrow), Wing length (blue arrow).

6.4.4 Data analysis

To investigate the effect of the population type, sex, and the interaction between population type and sex on each body measurement, we conducted a series of linear models in R (ver 4.1.3) (R Core Team 2022). One model was constructed for each of the seven measurements. We then used the emmeans package (Lenth 2022) to examine pairwise comparisons between the groups for type and sex. One skull measurement from the museum specimens was removed due to an error in data entry, since the recorded value was lower than could reasonably be expected compared to other birds (<15mm). Wing measurements were compared only between zoo and avicultural birds, as it was not possible to measure natural wing length in the museum skins. Therefore, any comparisons would not be meaningful. In some cases, both wings were damaged, and so the measurement taken is unlikely to reflect the bird's wing length accurately. Where both wings were reported as damaged, individuals were excluded from analyses for wing length only (n= 6)

6.4.5 Ethics Statement

Measurement of live birds was approved by The University of Manchester Animal Welfare and Ethics in Research Board.

6.5 Results

Birds differed both within and between populations for a number of the measurements examined. Here, we present meaningful pairwise comparisons, i.e., differences between sexes within each population and differences within sexes among populations (Table 6.1). Full information on pairwise comparisons can be found in Supplementary Information 6.1. The size of birds within sexes differed between populations. Avicultural males were larger than museum males across 5 of the 6 measurements, and the pattern reached marginal significance for beak length (Table 6.1; Figure 6.2). Similarly, avicultural males were larger than males from the zoo population for 2 of the 7 measurements and a similar pattern for beak height reached marginal significance (Table 6.1; Figure 6.2). However, for wing length, zoo males were larger than those from the avicultural population (Table 6.1; Figure 6.2). Zoo males were larger than museum males for 5 of the 6 measures (Table 6.1; Figure 6.2). Among females, avicultural females were larger than museum females for 3 of the 6 measures (Table 6.1; Figure 6.2). Zoo females were not significantly different in size to the avicultural population or museum population for any of the measures examined (Table 6.1; Figure 6.2).

Significant sexual size dimorphism was apparent in both the avicultural and zoo populations. In the avicultural population, males were larger than females for all measurements (Table 6.1; Figure 6.2). In the zoo population, males were larger than females for 4 of the 7 measurements (Table 6.1; Figure 6.2). Although patterns in other measures were in a similar direction, they did not reach marginal significance (Table 6.1; Figure 6.2). However, there was no evidence for sexual size dimorphism in the museum population – males and females did not differ significantly for any of the measures examined (Table 6.1; Figure 6.2). Populations also differed in the extent of sexual size dimorphism. Dimorphism in the museum population was significantly lower than for the avicultural population for 4 of the 6 measures (Table 6.2; Figure 6.2), with the final two measures reaching marginal significant (Table 6.2; Figure 6.2). Sexual size dimorphism was larger in the avicultural population compared to the zoo population for tarsus length and beak height (Table 6.2; Figure 6.2). Dimorphism in the zoo population was not significantly different from the museum population across all measures examined (Table 6.2; Figure 6.2).

Table 6.1: Pairwise comparisons between groups (bird's origin (Aviculture, Zoo, or Museum) and sex (male (M) or female (F)) for morphological measurements of Java sparrows. Only meaningful comparisons are presented (differences between sexes within populations and differences within sexes among populations). Full pairwise comparisons are presented in Supplementary Information 6.1. Significant differences are indicated in bold.

	Beak Length	Beak Width	Beak Height	Skull Length	Tarsus Length	Tail Length	Wing Length
Aviculture F – Museum F	-0.052 p=0.997	**0.228** **p=0.027**	0.246 p=0.207	-0.303 p=0.536	**0.962** **p<0.001**	**2.852** **p=0.011**	
Aviculture F – Zoo F	-0.316 p=0.103	0.156 p=0.407	-0.068 p=0.993	-0.213 p=0.892	0.327 p=0.658	1.714 p=0.453	-1.353 p=0.135
Aviculture F – Aviculture M	**-0.559** **p<0.001**	**-0.433** **p<0.001**	**-0.639** **p<0.001**	**-0.877** **p<0.001**	**-0.949** **p<0.001**	-**1.748** **p=0.039**	**-1.642** **p<0.001**
Museum F – Zoo F	-0.264 p=0.448	-0.073 p=0.976	-0.314 p=0.238	0.090 p=0.999	-0.635 p=0.135	-1.138 p=0.911	
Museum F – Museum M	-0.233 p=0.441	-0.161 p=0.414	-0.107 p=0.956	-0.427 p=0.314	-0.076 p=1.000	0.485 p=0.996	
Zoo F – Zoo M	**-0.453** **p=0.010**	**-0.359** **p=0.001**	-0.328 p=0.128	**-0.827** **p=0.002**	-0.263 p=0.877	-1.857 p=0.465	**-2.378** **p=0.003**
Aviculture M – Museum M	**0**.*275* *p=0.066*	**0.500** **p<0.001**	**0.778** **p<0.001**	0.147 p=0.946	**1.836** **p<0.001**	**5.086** **p<0.001**	
Aviculture M – Zoo M	-0.209 p=0.226	**0.229** **p=0.007**	0.*243* *p=0.093*	-0.163 p=0.894	**1.013** **p<0.001**	1.606 p=0.231	**-2.090** **p<0.001**
Museum M – Zoo M	**-0.484** **p<0.001**	**-0.271** **p=0.007**	**-0.534** **p<0.001**	-0.310 p=0.560	**-0.823** **p=0.001**	**-3.480** **p=0.001**	

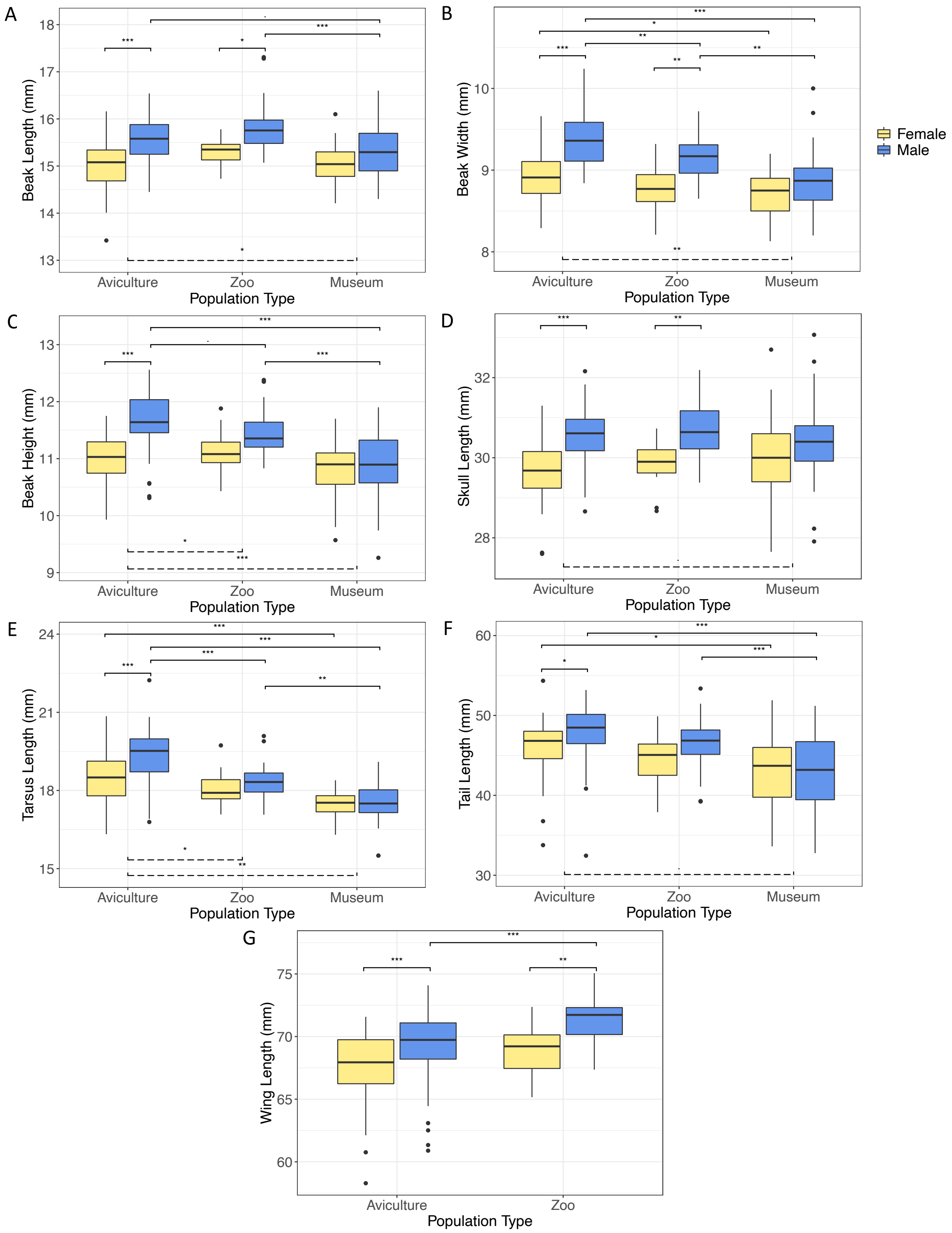

Figure 6.2: Comparisons of morphological measurements between avicultural, zoo and museum Java sparrows. Brackets indicate significant differences between groups (*** <0.001, ** <0.01, *<0.05, . <0.1). Solid lines represent pairwise differences between groups. Indications of significance are only presented for meaningful comparisons (i.e., between sexes within populations and among sexes between populations). Full details of pairwise comparisons can be found in

Supplementary Information 6.1. Dotted lines represent pairwise differences in dimorphism between populations.

Table 6.2: Pairwise comparisons for sexual size dimorphism between populations. Significant differences are indicated in bold and marginal significance is indicated by italics.

	Beak Length	Beak Width	Beak Height	Skull Length	Tarsus Length	Tail Length	Wing Length
Museum Dimorphism – Aviculture Dimorphism	**-0.326** **p=0.028**	**-0.271** **p=0.007**	**-0.532** **p<0.001**	*-0.450* *p=0.064*	**-0.873** **p=0.001**	*-2.234* *p=0.051*	
Zoo Dimorphism – Aviculture Dimorphism	-0.107 p=0.488	-0.074 p=0.479	**-0.311** **p=0.041**	-0.050 p=0.841	**-0.686** **p=0.013**	0.108 p=0.927	0.737 p=0.352
Museum Dimorphism – Zoo Dimorphism	-0.220 0.232	-0.198 0.111	-0.221 0.224	-0.400 0.184	-0.188 0.568	-2.342 0.100	

6.6 Discussion

We found significant differences in size and sexual dimorphism between museum, zoo, and avicultural birds. Both avicultural and zoo males were larger than museum males across most of the measures examined (five of six for each). Avicultural males were also larger than zoo males for two of the seven measurements, although zoo males had larger wings than avicultural males. Differences were less pronounced in females. Avicultural females were larger than those in museums for three of the six measures. There were no significant differences between zoo females and those in aviculture or museums. Males were larger than females in the avicultural (six of seven measures) and zoo (four of seven measures) populations. There were no significant differences between sexes in the museum population. Sexual size dimorphism was significantly larger in the avicultural population than the museum population (four of six measures), and for tarsus length and beak height compared to the zoo population, but did not differ significantly between museum and zoo populations.

Morphological change in captivity has been reported in other bird species (Soma 2005; Remeš & Székely 2010; Stojanovic et al. 2021). Changes in sexual size dimorphism have been less well explored (Soma 2005; Remeš & Székely 2010). Interestingly, the patterns observed in Java sparrows contrast with a close relative, the Bengalese finch (*Lonchura striata domestica*). As with Java sparrows, the domestic Bengalese finch was found to be larger than its wild strain, the white-rumped munia (*Lonchura striata*), although this was not confirmed statistically (Soma 2005).

However, the patterns of sexual size dimorphism were markedly different. In the white-rumped munia, males were significantly larger than females for both wing and tail length, although measures of tarsus and beak size were not significantly different between the sexes (Mizuta et al. 2003; Soma 2005). However, in the captive Bengalese finch, females were significantly larger than males for wing length, bill length, and bill width (Soma 2005). This is the opposite pattern to that found in Java sparrows, where males were larger than females in both captive populations (zoo and avicultural). Unlike Java sparrows, Bengalese finches are often used as foster parents for other bird species (Baptista 1978), and selection for parental care may have favoured increases in female body size.

As we used museum skins to represent the wild phenotype, it is important to consider the possible influence of post preservation shrinkage on our results. Shrinkage in museum skins has been reported for a broad range of bird species (e.g., Harris, 1980; Kuczynski et al., 2002; Wilson & McCracken, 2008; Winker, 1993). Different measures are likely to be differentially affected by shrinkage. Tarsus shrinkage was found to be insignificant for some bird species (Fjeldså 1980; Kuczynski et al. 2002), although some studies report shrinkage (Bjordal 1983; Winker 1993; Wilson & McCracken 2008), and one study reported an increase in measurements post preservation (Herremans 1984). Shrinkage in beak measurements is like to be affected by beak morphology and specific measurement. Most studies report some degree of shrinkage for beak measures (e.g., Harris, 1980; Kuczynski et al., 2002; Wilson & McCracken, 2008), although this is not true for all measures in all species examined and beak measures within a single species may show different levels of shrinkage (Harris 1980). In puffins (*Fratercula arctica*), although beak length declined during early preservation, beak depth did not show a significant decrease (Harris 1980). For tail length, both positive (Bjordal 1983; Herremans 1984) and negative change (Winker 1993; Kuczynski et al. 2002; Wilson & McCracken 2008) post preservation have been reported. The fact that similar differences, albeit to a smaller extent, are present between zoo and avicultural birds suggests a tangible role of the captive environment on Java sparrow morphology, as these birds will not have been affected by shrinkage due to museum preservation. Differences in sexual size dimorphism between museum and captive birds will only be affected if male and female museum specimens are differentially affected by shrinkage. In general, this has not been examined in depth for most species, although Wilson & McCracken (2008) report some differences in shrinkage correction factors for male and female cinnamon teal (*Spatula cyanoptera*). However, for some measures, we report large differences between males and females in captive collections, and it seems unlikely that these would have been eliminated by small differences in shrinkage levels reported in previous studies. In order to fully understand the effect of shrinkage on the comparisons presented in the study, it is necessary to compare captive populations with contemporary wild caught birds.

There are several potential explanations for the morphological differences observed between Java sparrow populations. Firstly, changes in size in captive populations could be a plastic response mediated by environmental differences between wild and captive settings. In captive mammals, diet has been implicated in changes in cranial morphology. In a meta-analysis of the cranial morphology of captive mammals, carnivorous species significantly differed from their wild counterparts in skull length, width and length to width ratio (Siciliano-Martina et al. 2021a). Dietary breadth was also an important feature, with species with a narrower dietary niche more likely to differ from their wild counterparts (Siciliano-Martina et al. 2021a). Early life diet can affect nestling growth in birds, with permanent effects on adult size (Boag 1987; Johnston 1993). In zebra finches (*Taeniopygia guttata*), young birds fed a high-quality diet grew at a faster rate than those on low quality diets, and this difference persisted into adulthood (Boag 1987). However, there was no evidence for an interaction between diet and sex. As such, whilst diet could affect the size differences between populations observed in our study, it may not fully explain the patterns of sexual dimorphism in Java sparrows. Husbandry routines, such as regular cleaning, may eliminate certain stressors in captive environments. In particular, a reduction in parasite prevalence may explain differences in size and sexual dimorphism in captivity. Parasite load influences growth and development in a number of bird species, with high parasite loads restricting nestling size (e.g., Fessl et al., 2006; Potti & Merino, 1996). As such, a lower prevalence of parasites in captive environments may result in bigger birds. Additionally, parasite load could also contribute to the patterns of sexual dimorphism observed in Java sparrows. In pied flycatchers *(Ficedula hypoleuca*), a significant interaction of sex and parasite load was found with respect to size; males reared in nests with high parasite loads were affected by infestations, facing a greater reduction in size than females (Potti & Merino 1996). In this way, increased parasite loads in wild populations could reduce sexual dimorphism due to males being more affected by the stressor. The reduction of the stressor in captive populations allows for sexual dimorphism to be expressed.

Alternatively, morphological changes in captivity could be a result of selection during captive breeding. Many domestic species are subject to artificial selection, with specific traits being selected by humans based on desired traits, which can have a significant effect on morphology (e.g., Drake & Klingenberg, 2010; Pelletier et al., 2021; Remeš & Székely, 2010). It is therefore possible that artificial selection in avicultural Java sparrows could result in increased body size. Changes in body size may also be related to genetic correlations with other traits under artificial selection. For example, male house mice selectively bred for high voluntary wheel-running behaviour had smaller body mass and larger testes than control lines (Klomberg et al. 2002). However, artificial selection is unlikely to fully explain the patterns we found in this study, even if it occurs in avicultural populations. We found similar changes in both the avicultural and zoological populations. Artificial selection is generally avoided in conservation breeding programmes in zoos, as these programmes follow a range of guidelines regarding genetic diversity (Frankham 2008;

Williams & Hoffman 2009; Hvilsom et al. 2022). In addition, pairs were not assigned in the zoological population examined in this study, further reducing the likelihood form human-induced artificial selection.

A more likely scenario is inadvertent selection in captivity due to altered selection pressures in the captive environment, such as a lack of predators (Williams & Hoffman 2009). Dietary differences between wild and farmed mallards (*Anas platyrhynchos*) influenced selection pressures for beak morphology (Champagnon et al. 2010); larger food items in captivity produced a relaxation of selective pressures promoting fine-sieved bills resulting in a reduction in lamellar density. In line with this, lamellar density in wild mallard populations decreased over time, which was most likely explained by introgression with captive birds that had been released to supplement the population (Champagnon et al. 2010). Whilst differences in beak morphology could be explained by plastic responses to food availability, evidence of introgression suggests selection and a genetic component to phenotypic differences. Phenotypic and genetic change were investigated in detail in the Houbara bustard (*Chlamydotis undulata*) captive breeding programme (Chargé et al. 2014). Body mass increased over generations in captivity for both males and females, and these phenotypic changes reflected genetic changes in the population. Male and female bustards showed different levels of change, suggesting that inadvertent selection could also contribute to sex differences in morphology. Interestingly, there was no evidence for direct selection on body mass, although there was for reproductive traits. Changes in breeding values for body mass were likely the result of genetic correlations with other traits under strong selection (Chargé et al. 2014). Even though selection may not be acting directly on morphology, inadvertent selection acting on other traits could influence the morphological phenotype. However, a similar, progressive change in body mass was not found during captive breeding of the orange-bellied parrot (*Neophema chrysgaster*) (Stojanovic et al. 2020), although wing morphology differed between captive birds and museum specimens (Stojanovic et al. 2021). The effects of adaptation to captivity through inadvertent selection are unlikely to be universal across species, and are likely to depend on the species' life history and captive conditions. Given the differences in selection pressures between wild and captive environments, it seems likely that some inadvertent selection could have occurred in captive Java sparrows. However, it is not clear whether this selection acts on morphology, a linked trait, or is unrelated to morphological change. It is difficult to determine the extent of inadvertent selection without a full understanding of the genetic background of all populations, which is the case for out study. Even where the genetic background is understood, it is possible that after long periods of captive breeding, effects of inadvertent selection may reduce as captive optima are reached (Gilligan & Frankham 2003), making the signatures of inadvertent selection more difficult to detect.

Sexual selection may also play a role in both increased size and changes in sexual size dimorphism in captive environments. Preferences for large bodied individuals could result in increased size and sexual dimorphism in captivity. When examining sexual size dimorphism across groups, species often adhere to Rensch's rule, the tendency for sexual size dimorphism to increase with body size in lineages where males are the larger sex (Székely et al. 2004; Ceballos et al. 2013; Adams et al. 2020; Caspar & Begall 2022). Although not generally examined, a similar process may occur at the level of populations within a species. One explanation for this pattern is sexual selection on males for large body size, resulting in increased male size and a smaller, correlated increase in female size (Abouheif & Fairbairn 1997; Dale et al. 2007). There is some evidence for preference of large males in Java sparrow females (Hasegawa et al. 2011), and this could drive increases in body size within populations. Even where females are paired with a mate, as in avicultural populations, females may be able to exert sexual selection by differential allocation to preferred mates, which has been reported in the closely related Bengalese finch (Soma & Okanoya 2013). In addition, larger males were found to have higher song performance (Kagawa & Soma 2013). In this species, songs are solely used in a courtship context, so increased performance with body size may result in indirect selection for body size, although specific preference for song features has not been reported in the Java sparrow. In wild environments, males and females experience natural and sexual selection, which results in an overall net selection which may, or may not, differ between sexes and contribute to sex differences (Cox & Calsbeek 2009). In captivity, changes in the environment and selection pressures experienced by Java sparrows may enable individuals to grow to a larger size than would be possible in wild environments, allowing greater expression of sexual size dimorphism.

Changes in morphology in the Java sparrow could influence conservation efforts, resulting in captive-bred individuals having decreased fitness in wild environments. Changes in morphology, particularly changes in beak morphology, may result in altered foraging efficiency and dietary niche in captive individuals. In the large cactus finch (*Geospiza conirostris*), beak-shape variation, particularly in beak length and depth, influenced the birds' ability to forage on certain food items, and this affected fitness under changing environmental conditions (Grant & Grant 1989). Due to changes in sexual size dimorphism, males may suffer a greater reduction in performance in wild environments as they are larger than females, therefore showing greater deviation from the wild-type phenotype. Morphological changes may also influence vocal communication in this species. In many birds, changes in beak morphology and body size can influence the production of vocalizations; large body size is often associated with lower vocal frequencies and larger beaks are often associated with slower tempo of vocalizations (Derryberry et al. 2018; García & Tubaro 2018; Friedman et al. 2019). In Java sparrows, body size is associated with increased vocal performance (Kagawa & Soma 2013), although changes in other parameters have not been investigated. If differences in morphology are solely related to plasticity in response to environmental factors, such as improved conditions in captivity, the impact of morphological differences may not persist over multiple generations when birds experience wild conditions. However, the impacts may still persist for a short period. In Japanese quail, females receiving a higher quality diet produced more eggs, which were larger in size, to those receiving a low-quality diet (Grindstaff et al. 2005). This suggests that birds from captive environments may experience improved reproductive success when initially moved to a wild environment. However, if morphological changes are related to selection, release of captive individuals may have a significant effect on the wild population. Introgression with the wild population may result in propagation of captive adaptations, altering the phenotype in the wild populations (Lynch & O'Hely 2001; Champagnon et al. 2010, 2012). Quantitative genetic models suggest that even low levels of gene flow from captive to wild populations during supportive breeding can shift the mean phenotype of wild populations towards the captive optimum (Ford 2002). This could result in reduced performance in wild populations, negatively impacting conservation efforts. Problems related to introgression could be exacerbated in Java sparrows due to sexual selection; if wild females prefer the larger, captive males, captive-born males may have greater reproductive success than smaller, wild-type males.

Overall, we found that Java sparrows in captive (zoo and avicultural) environments were larger, particularly in males, and had more pronounced sexual size dimorphism than museum specimens across a range of measures. These findings suggest that Java sparrows in captive environments show signs of adaptation to captivity, which could influence future conservation efforts. From our data, it is not possible to determine the precise influences affecting body size in captive environments, but it seems likely that environmental factors, such as improved diet, inadvertent selection for morphology or other traits, and sexual selection may all play a role in influencing morphological phenotype. The relative contribution of these mechanisms will influence the consequences of these phenotypic differences during conservation, with higher contributions from selection increasing the possible negative consequences. To fully understand the effects of adaptation to captivity in this species, future research should aim to unpick the roles of each mechanism to further our understanding and allow for appropriate mitigation to be put in place.

6.7 Author Contributions

RL, LW and TG conceptualized the study. RL was responsible for data collection. RL, TG, and SS contributed to data analysis. RL wrote the initial draft of the manuscript, with all authors contributing to reviewing and editing the manuscript. LW, SS, and TG provided supervision during the project.

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6.10 Supplementary Information

Supplementary Information 6.1: Full pairwise comparisons between groups (bird's origin (Aviculture, Zoo, or Museum) and sex (male (M) or female (F)) for morphological measurements of Java sparrows. Significant differences are indicated in bold. Marginal significance is indicated by italics.

	Beak Length	Beak Width	Beak Height	Skull Length	Tarsus Length	Tail Length	Wing Length
Aviculture F – Museum F	-0.052 p=0.997	**0.228** **p=0.027**	0.246 p=0.207	-0.303 p=0.536	**0.962** **p<0.001**	**2.852** **p=0.011**	
Aviculture F – Zoo F	-0.316 p=0.103	0.156 p=0.407	-0.068 p=0.993	-0.213 p=0.892	0.327 p=0.658	1.714 p=0.453	-1.353 p=0.135
Aviculture F – Aviculture M	**-0.559** **p<0.001**	**-0.433** **p<0.001**	**-0.639** **p<0.001**	**-0.877** **p<0.001**	**-0.949** **p<0.001**	**-1.748** **p=0.039**	**-1.642** **p<0.001**
Aviculture F – Museum M	-0.284 p=0.054	0.067 p=0.919	0.139 p=0.722	**-0.729** **p<0.001**	**0.887** **p<0.001**	**3.338** **p<0.001**	
Aviculture F – Zoo M	**-0.768** **p<0.001**	**-0.203** **p=0.020**	**-0.396** **p<0.001**	**-1.040** **p<0.001**	0.064 p=0.999	-0.142 p=1.000	**-3.732** **p<0.001**
Museum F – Zoo F	-0.264 p=0.448	-0.073 p=0.976	-0.314 p=0.238	0.090 p=0.999	-0.635 p=0.135	-1.138 p=0.911	
Museum F – Aviculture M	**-0.507** **p<0.001**	**-0.661** **p<0.001**	**-0.885** **p<0.001**	**-0.574** **p=0.017**	**-1.911** **p<0.001**	**-4.601** **p<0.001**	
Museum F – Museum M	-0.233 p=0.441	-0.161 p=0.414	-0.107 p=0.956	-0.427 p=0.314	-0.076 p=1.000	0.485 p=0.996	
Museum F – Zoo M	**-0.717** **p<0.001**	**-0.432** **p<0.001**	**-0.642** **p<0.001**	**-0.737** **p=0.004**	**-0.898** **p<0.001**	**-3.00** **p=0.021**	
Zoo F – Aviculture M	-0.244 p=0.338	**-0.589** **p<0.001**	**-0.571** **p<0.001**	**-0.664** **p=0.011**	**-1.276** **p<0.001**	**-3.463** **p=0.004**	-0.288 p=0.967
Zoo F – Museum M	-0.031 p=1.000	-0.089 p=0.932	0.207 p=0.645	-0.516 p=0.200	0.560 p=0.207	1.624 p=0.645	
Zoo F – Zoo M	**-0.453** **p=0.010**	**-0.359** **p=0.001**	-0.328 p=0.128	**-0.827** **p=0.002**	-0.263 p=0.877	-1.857 p=0.465	**-2.378** **p=0.003**
Aviculture M – Museum M	**0**.*27* *p=0.066*	**0.500** **p<0.001**	**0.778** **p<0.001**	0.147 p=0.946	**1.836** **p<0.001**	**5.086** **p<0.001**	
Aviculture M – Zoo M	-0.209 p=0.226	**0.229** **p=0.007**	0.*243* *p=0.093*	-0.163 p=0.894	**1.013** **p<0.001**	1.606 p=0.231	**-2.090** **p<0.001**
Museum M – Zoo M	**-0.484** **p<0.001**	**-0.271** **p=0.007**	**-0.534** **p<0.001**	-0.310 p=0.560	**-0.823** **p=0.001**	**-3.480** **p=0.001**	

Chapter 7: The effect of prolonged zoo closure on the sound environment in zoo aviaries

Rebecca N Lewis, Leah J Williams, Selvino R de Kort, Susanne Shultz, R Tucker Gilman

7.1 Chapter Summary

To fully understand how mating traits, such as vocalizations, can change during ex situ breeding programmes, it is important to investigate the potential drivers of change in the captive environment. In Chapter 6, I examined the differences in size and sexual dimorphism between wild and captive populations, which could contribute to changes in vocal behaviour, as well as affecting animals' suitability for release. Another possible driver of vocal change in the captive environment is the captive soundscape. The sound environment experienced by animals in captivity is likely to differ from that in in situ environment (Lara & Vasconcelos 2019), particularly in terms of anthropogenic influences. These differences may result in the masking of important vocal signals in the captive environment (Brumm & Slabbekoorn 2005; Blickley & Patricelli 2010). As a result, birds may alter vocal behaviour to avoid masking and improve communication efficacy (Brumm & Slabbekoorn 2005). These changes are often reported in birds in urban environments, which sing at higher amplitudes and higher frequencies than those in rural environments (Slabbekoorn et al. 2003; Nemeth & Brumm 2009).

The study of sound in zoos has lagged behind that in in situ environments (Clark & Dunn 2022) and the acoustic environment is still one of the least well studied aspect of the zoo (Binding et al. 2020). Despite this, sound in zoos has a number of potential applications, such as improving animal welfare and informing management decisions. As well as more traditional studies of sound levels, a number of metrics for measuring the sound environment have been developed for use in in situ settings (Sueur et al. 2014; Clark & Dunn 2022) that may be useful in describing the zoo soundscape.

The COVID-19 lockdowns in 2020 provided a unique opportunity to examine the effect of humans on zoo soundscapes due to prolonged zoo closures. In this chapter, I examined the effects of lockdown on the sound environment of three mixed-species aviaries to determine the impact of human presence. I measured sound pressure levels, which are often used to understand sound in zoos, and also investigated the potential use of acoustic indices, which are usually used in situ, to characterize the zoo soundscape. Understanding how humans impact the sound environment in zoos can help us to determine how sounds experienced by animals in zoos may differ from those in the wild. Although interest in the sound environment in zoos has grown, this study represents one of the first in-depth investigation into human impacts on the zoo soundscape using a range of measurements, including acoustic indices.

7.2 Abstract

Captive environments differ from those in situ in a number of ways, the most obvious being the consistent human presence in zoos. Whilst the effect of human presence on animal behaviour and welfare has been widely examined, its effect on other aspects of the zoo environment is less often considered. In particular, human presence may influence the sound environment in zoos in a number of ways e.g., through speech, footfall, and machinery. The COVID-19 lockdown in 2020 provided a unique opportunity to study zoo soundscapes in the absence of visitor presence. We compared the sound environment during the period of zoo closure in 2020 to a period of normal zoo opening during 2019 across three zoo aviaries. We examined wide band frequency measures of sound pressure levels as well as sound pressure levels in narrower, defined frequency bands. In addition, we explored the use of acoustic indices, specifically the Acoustic Complexity Index (ACI) and the Normalized Difference Soundscape Index (NDSI), to describe zoo soundscapes. We found a significant effect of human presence on the sound environment, with aviaries being generally quieter and with a lower proportion of anthropogenic sound during the 2020 zoo closure period. We found that NDSI may be a particularly useful index for describing the soundscape in zoos, although further information on how it is influenced by human speech would be beneficial. Overall, we recommend that researchers interested in examining the sound environment in zoos use a range of measures, including sound pressure levels and acoustic indices, to provide a holistic overview of the zoo soundscape.

7.3 Introduction

Captive environments differ from those in situ in a number of aspects, such as the lack of predators, reductions in disease and parasites, and space constraints (Frankham 2008). Perhaps the clearest differences between the environment in zoos and those in situ is the consistent presence of human influences. The presence of human visitors (and human caretakers) on animal behaviour and welfare has been widely examined (Hosey 2000, 2008; Davey 2007; Fernandez et al. 2009; Sherwen & Hemsworth 2019). However, the effect of chronic human presence of other aspects of the environment is less often considered.

Human presence can influence the sound environment experienced by animals in zoos. In general, sound sources in zoos can be separated into four broad categories: 1) permanent sources of sound, such as heating, ventilation, and air conditioning (HVAC) systems; 2) temporary sound from maintenance, construction, and other events; 3) human speech and footfall; and 4) sounds produced by other animals (Clark & Dunn 2022). Three of these four categories are related to human presence and anthropogenic sound sources, suggesting that the constant presence of humans is likely to have a significant impact on the sound environment. As such, animals in captivity may experience sound environments that differ to those in situ (Lara & Vasconcelos 2019). In addition, the contributions of different sound sources are likely to differ between zoos and enclosures, and, therefore, sound environments are also likely to vary among captive environments (Clark & Dunn 2022).

The sound environment is an important consideration in captivity due to its potential effects on animal behaviour, communication, and welfare. Increases in sound from visitors have been associated with a wide range of behavioural changes across taxa, which are often interpreted as negative (reviewed in Sherwen & Hemsworth, 2019). As well as potential effects on animal behaviour, exposure to environmental sound can also affect physiology, development, neural functions, and genetics (Kight & Swaddle 2011). Importantly for ex situ breeding programmes, sound can also negatively affect reproductive success (Halfwerk et al. 2011; Kight et al. 2012). Increased background sound can also obscure sounds that are important for survival and reproduction, an effect known as masking, which could reduce communication efficacy (Brumm & Slabbekoorn 2005; Barber et al. 2010; Blickley & Patricelli 2010). Many taxa are reported to alter vocal behaviour to reduce the effect of masking (Brumm & Slabbekoorn 2005; Slabbekoorn & Ripmeester 2008). As such, the sound environment in captivity may alter vocal behaviour, contributing to vocal divergence between populations, which may have knock-on effects for conservation programmes (Passos et al. 2017; Lewis et al. 2021). Given its potential effects on animal welfare and conservation, understanding the sound environment has important implications for animal management in captivity.

Despite its importance, the study of sound in zoos has lagged behind that in in situ environments (Clark & Dunn 2022). The present literature has a significant focus on measuring maximum sound pressure levels of environmental sound (Clark & Dunn 2022). This tells us about the level of environmental sound, but does not provide any additional information about the characteristics of the sound environment overall i.e., what that sound is like. However, there is increasing interest in sound in zoological collections (Pelletier et al. 2020; Rose et al. 2021; Clark & Dunn 2022). Due to recent technological advances, such as the development of low-cost autonomous recording units (ARUs), it is possible to collect large amounts of data with comparatively little effort (Brandes 2008). This allows for a more in-depth study of the sound environment beyond traditional sound levels, such as the investigation of sound in specific frequency bands (Pelletier et al. 2020). There also is ample opportunity to use techniques developed for use in situ in captivity, such as the principles of soundscape ecology (Pijanowski et al. 2011) and acoustic indices (Sueur et al. 2014; Bradfer-Lawrence et al. 2019; Clark & Dunn 2022). Acoustic indices reduce large scale data into a single metric which provides information about the characteristics of sound in the environment, such as complexity or diversity, not just its intensity (Sueur et al. 2014). Although sound pressure level, which provides a quantification of the intensity of environmental sound, is an important measure for examining the sound environment and its effects on animals in zoos, combining additional measures, such as acoustic indices can give a more well-rounded view of sound in zoos, which may be beneficial for management.

As zoos are rarely closed for prolonged periods, visitors are present throughout the year. As such, it is difficult to determine the effects of human presence on the sound environment, as they are constantly present during the day when many animals are active. The COVID-19 lockdown in 2020 provided a unique opportunity to study sound in the zoo in the absence of visitors, as well as with reduced influences from other anthropogenic sound sources e.g., road and air traffic. A number of studies have examined the effect of lockdown and the absence of visitors on animal behaviour (Carter et al., 2021; Finch et al., 2022; Jones et al., 2021; Masman et al., 2022; Podturkin, 2022; Williams et al., 2021b, 2021a, 2022), but, to our knowledge, the effect on the sound environment has not been explored. We examined the sound environment in three mixed species aviaries, two outdoor and one indoor, at Chester Zoo in April – May of 2019 and 2020 to explore the effect of human presence. We examined sound pressure levels using wide frequency band measures, as well as in narrower, distinct frequency bands. In addition to sound pressure levels, we also examined two acoustic indices. Firstly, we examined the Acoustic Complexity Index (ACI) (Pieretti et al. 2011), which measures the complexity of the soundscape, and has been found to correlate with the number of avian vocalizations in the environment. Secondly, we examined the Normalized Difference Soundscape Index (NDSI) (Kasten et al. 2012), which quantifies soundscape naturalness by comparing the relative intensities of anthropogenic and biotic sound in the environment.

7.4 Methods

7.4.1 Data collection sites and periods

Data were collected from three aviaries at Chester Zoo, UK. The Bali Temple aviary is an outdoor, walkthrough aviary located near the edge of the zoo, close to a main road (A41). The Sumatra aviary is also an outdoor, walkthrough aviary, which is located further into the zoo grounds, further from external roads. In a walkthrough aviary, the visitor path is located inside the aviary, i.e., visitors and birds occupy the same space without barriers. The Dragons in Danger aviary is an indoor aviary located near the centre of the zoo. Although this aviary is not a walkthrough, the visitor path moves through the building with netted aviaries either side of the walkway, meaning that visitors walk through the middle of the bird habitat. Sound recordings were made between April 30th and May 21st in 2019 and April 8th and May 8th in 2020. Further details of the aviaries and recording dates can be found in Table 7.1. All aviaries contained a range of bird species, including various passerines, pigeons, pheasants and parrots, many of which are native to South East Asia (species lists for each aviary in each time period can be found in Supplementary Information 7.1).

Table 7.1: Aviaries sampled and dates of recording during the study

Aviary	Type	2019 Recording Dates	2020 Recording Dates
Bali Temple	Outdoor, walkthrough	30/04/19 – 07/05/19	08/04/20 – 12/04/20
Sumatra Aviary	Outdoor, walkthrough	07/05/19 – 14/05/19	22/04/20 – 28/04/20
Dragons in Danger	Indoor, not walkthrough	14/05/19 – 21/05/19	30/04/20 – 08/05/20

7.4.2 Data collection method

Sound recordings were made using Wildlife Acoustics SM4s (Wildlife Acoustics Inc., Maynard, MA, USA) placed within the enclosure. For the Dragons in Danger aviary, the recording device was placed in a central location within the bird area, although this was outside of the netted aviaries. Devices were set to record continuously across the days, and continued to record until either the batteries had run out or the memory card had reached capacity. We used a 24 kHz sampling rate for recordings. This allowed us to capture information on sounds below 12 kHz, which is relevant to birds, as biotic sound (and the frequency limits of many bird songs) is typically concentrated below 8 kHz (Slabbekoorn & Ripmeester 2008; Pijanowski et al. 2011; Kasten et al. 2012). This sampling rate, therefore, allowed us to make relevant sound recordings whilst preserving space on memory cards and battery life of recording units.

7.4.3 Data extraction

Data were extracted using Kaleidoscope Pro 5.4.7 (Wildlife Acoustics Inc., Maynard, MA, USA). For each of the aviaries, we extracted information on sound pressure levels and acoustic indices for comparison.

Firstly, we extracted the mean (L_{eq}) sound pressure level (SPL) for each 1-hour period during the day using Kaleidoscope Pro. Mean sound pressure level is a representative measure for sounds that remain more or less constant over time, and is less affected by sharp bursts of sound that may occur e.g., slamming doors. Sound pressure levels used the standard reference of 20uPa, where 1 Pa is equal to a sound pressure level of 94 dB.

7.4.3.1 Wide frequency band measures

We extracted two wide frequency band measurements of sound pressure level using Kaleidoscope Pro, A-weighted decibels (dB(A)) across the recording, and Z-weighted decibels (dB(Z)) between 10 – 10000 Hz. A-weighted sound level measurements apply a filter adjusted to the human hearing range with reduced weighting of low and high frequency sound (Kurra 2021). A-weighted measurements are often used in in acoustic studies in zoos, as most commercial dB readers use this metric. Therefore, we included dB(A) values to allow for comparison with other studies. Moreover, birds' hearing range is broadly similar to that of humans (Catchpole & Slater 2008) suggesting that dB(A) may be meaningful when examining sound in an aviary setting. Z-weighted dB uses a flat-frequency response, where all frequencies are given equal weight, providing a less anthropocentric measure of sound in the environment. As such, dB(Z) provides an objective measure of sound in the environment.

7.4.3.2 Defined frequency band measures

We also examined sound pressure levels in different frequency bins. We extracted information on sound pressure levels from 30 third octave bands (central frequencies 19.7 – 10079.4 Hz) using Kaleidoscope Pro. We then combined the sound pressure level (SPL) from these bands into larger frequency 'bins' for analysis (dB(Z)), based on their relevance for species and sound in zoological collections, using the following equation (Lin et al. 2021):

$$\text{SPL}_{\text{total}} = 10\log(10^{\frac{SPL1}{10}} + 10^{\frac{SPL2}{10}} + \cdots)\ \text{dB}$$

We chose combined frequency bands to align with those calculated in Pelletier et al. (2020), as consistency in methodology between studies is useful for comparisons and increasing repeatability (Clark & Dunn 2022). However, there are some differences. We did not study ultrasounds, as these are unlikely to be relevant to birds. In addition, we did not study sounds above 12 kHz. Most biotic sound is prevalent between 2-8 kHz (Pijanowski et al. 2011; Kasten et al. 2012), and most birds vocalize within this range, although some species have higher vocalizations (Slabbekoorn & Ripmeester 2008). As such, frequencies below 12 kHz are likely to be most relevant for birds. For this reason, our high frequency band spans a smaller frequency range than that described in Pelletier et al. (2020) (8979.7 – 17, 959 Hz). Our categories were therefore defined as follows: very low frequency (17.6-111.4 Hz); low frequency (111.4 – 890.9 Hz); mid frequency (890-9 – 8979.7 Hz); and high frequency (8979.7 – 11313.7 Hz). These divisions are meaningful for the study of sound in aviaries and elsewhere in zoological environments. The very low frequency band covers sound below 100 Hz, including infrasound (<20 Hz). Infrasound has a number of reported effects on health in both humans and animals (Persinger 2014; Lousinha et al. 2018; Pereira et al. 2021). In addition, frequencies below 100Hz have been suggested to be more relevant to human health than infrasound alone (Broner 1978), and this may also be true in non-human mammals and other taxa (Pelletier et al. 2020). Many bird taxa, such as doves, corvids, woodpeckers, and herons often use frequencies below 1 kHz for communication (Slabbekoorn & Ripmeester 2008). Therefore, sound in the 'Low' frequency band may be particularly relevant for these species, both due to their vocal range and the potential for increased sound to mask vocalizations. The mid frequency band is biologically relevant for many species, and covers a large proportion of biotic sounds. Most mammals have their highest hearing sensitivity between 1 and 8 kHz (Fay, 1988). Similarly, most birds hear best between 1 and 5 kHz, with greatest sensitivity between 2 and 3 kHz (Dooling 1992; Dooling et al. 2000). However, many songbirds use frequencies of up to 8 kHz in their songs (Slabbekoorn & Ripmeester 2008). In addition, biological sounds are most common between 2 and 8 kHz (Pijanowski et al. 2011; Kasten et al. 2012). Finally, the high frequency band covers higher frequency biotic sound, which may include some avian vocalizations.

7.4.3.3 Acoustic indices

The study of soundscape ecology, which examines all sounds emanating from a given landscape considers many characteristics of the sound environment other than sound levels (Pijanowski et al. 2011). Acoustic indices are statistics that can be used to summarize aspects of the soundscape and reduce complex information into more manageable data (Sueur et al. 2014; Towsey et al. 2014). Acoustic indices are not currently used in captive animal environments (Clark & Dunn 2022). However, they can provide useful information on the soundscape, such as complexity or naturalness, that can be used to understand sound in zoos beyond sound levels. A wide range of indices are available (Sueur et al. 2014; Bradfer-Lawrence et al. 2019), however, not all are relevant in captive settings. We identified two indices, detailed below, that may be particularly relevant to the zoo environment to examine how they behave in the different aviaries and situations in this study. We used the standard settings, as outlined in the original papers, during extraction (Pieretti et al. 2011; Kasten et al. 2012). Whilst acoustic indices can be tuned to match individual habitats, we chose to use the standard measures for two reasons. Firstly, acoustic indices are yet to be used in zoological settings, so it is difficult to determine how default settings perform and, if sub-optimally, how they should be adjusted. Secondly, there is a need for consistent measurements across zoos so that comparisons can be made. Currently, methods for measuring sounds in captive collections are inconsistent (Clark & Dunn 2022), making comparisons and replication difficult. Using the default settings for acoustic indices means that they are easily applied by researchers in zoos, who may not have significant technical knowledge to appropriately adjust the parameters of the indices.

The first index we extracted is the Acoustic Complexity Index (ACI) (Pieretti et al. 2011), a measure of soundscape complexity. The ACI is based on the observation that many biotic sounds are intrinsically variable, whilst human generated sound is often more constant in intensity (Pieretti et al. 2011; Wolfenden et al. 2019). As such, it has the potential to capture information about biotic sound without the effect of anthropogenic sound. Briefly, the index compares amplitude differences between time intervals within narrow frequency bands, and these measures are combined to give the overall index (Pieretti et al. 2011). Variable soundscapes, e.g., those with high biophony (sound of biotic origins e.g., birds), have high ACI values (Pieretti et al. 2011). In situ, ACI values have been found to correlate with the number of bird vocalizations in the environment (Pieretti et al. 2011). Therefore, ACI could be used in captive settings to gauge vocal activity within enclosures without the need to individual count vocalizations in person or from recordings. We used the entire frequency range of the recording to calculate the ACI, as no minimum frequency was set in the original description (Pieretti et al. 2011), and our maximum frequency is dictated by the sampling rate. We used an FFT window size of 512, as in the original description of the index (Pieretti et al. 2011).

The second index we extracted is the Normalized Difference Soundscape Index (NDSI), which provides a measure of soundscape 'naturalness' (Kasten et al. 2012). The NDSI represents the relative ratio of anthropogenic compared to biotic sound, and can be used to estimate the level of anthropogenic disturbance on the soundscape (Kasten et al. 2012). To achieve this, sound in different frequency bands of the recording is compared. In this way, the NDSI could provide a useful metric to assess the dominance of anthropogenic sounds in captive environments, and this information could be used to inform necessary sound mitigation. Mechanical sounds are generally prevalent between 1 and 2 kHz, whereas biological sounds are more commonly found between 2 and 8 kHz (Pijanowski et al. 2011; Kasten et al. 2012). As such, these bands are used to represent anthropogenic and biotic sound in the index. Briefly, the NDSI calculates the power spectral density of the signal and estimates are computed for each of the specified frequency ranges, which are then compared (Kasten et al. 2012). As in the original study, we used a 1 – 2 kHz frequency range to represent anthropogenic sound and a 2 – 8 kHz frequency range to represent biotic sound. The NSDI returns a value between -1 and +1, with positive values indicating relatively more biotic compared to anthropogenic sound and negative values indicating relatively more anthropogenic compared to biotic sound (Kasten et al. 2012). Some sound in our recordings, particularly at low frequencies, may fall outside of this range, but we chose to use the default values for acoustic indices. We used an FFT window size of 512. Whilst this was not specified in the original study (Kasten et al 2012), this window size is common in bioacoustics research and matches with that used for the ACI.

For each index, we used Kaleidoscope Pro to extract index values for each 60 s section of recordings, using the settings outlined above. An average of each index was then taken per hour to be used in analyses. This allowed us to examine comparable timeframes even though recordings taken in different aviaries had different start times.

7.4.4 Data analysis

To examine the effect of year on features of the sound environment we used a series of Generalized Additive Models (GAMs) implemented in the mgcv package (Wood 2011) in R 4.1.2 (R Core Team 2022). The use of GAMs allows us to account for the non-linear effect of time on the sound environment by fitting a spline to the time variable. As aviaries are expected to behave differently both within and between years, the set of models described below were run separately for each aviary in the dataset. This aids interpretation of the results by removing high order interactions between factors. All models included year and time as predictors, as well as date as a random effect. In each case, year was included as a factor in the model, comparing sound during the 2020 zoo closure period to the period of zoo opening in 2019. Time was included in models as a spline to allow for its non-linear effect on sound. Including date as a random variable allows us to account for non-independence of data points within days, and that days may intrinsically differ. For analyses examining defined frequency bands the models also included the frequency band of the measurement (as a factor) and the interaction between frequency band and year.

7.5 Results

7.5.1 Wide frequency band measures

Wide frequency band measures of sound pressure levels varied significantly between years, although effects differed between aviaries. When examining dB(A), sound pressure levels (SPL) in both the Dragons in Danger and Sumatra aviaries were quieter in 2020 than 2019 (Table 7.2; Figure 7.1A), and the effect was most pronounced in Dragons in Danger. There was no significant difference in dB(A) between years in the Bali Temple aviary (Table 7.2; Figure 7.1A). For dB(Z), all aviaries were significantly quieter in 2020 than 2019 (Table 7.2; Figure 7.2A). Across all models, the spline for time was significant ($p<0.01$), meaning that sound pressure levels varied significantly over the course of the day.

Table 7.2: Generalized Additive Models (GAMs) examining the effect of year (as a factor) on mean sound pressure level (SPL) in dB(A) and dB(Z) in three zoo aviaries (Bali Temple, Dragons in Danger and Sumatra). Estimates represent the difference in 2020 compared to 2019

	Mean Sound Pressure Level (dB(A))		Mean Sound Pressure Level (dB(Z))	
	Year (2020)		Year (2020)	
	Estimate	p-value	Estimate	p-value
Bali Temple (2020)	3.726	0.119	-5.481	<0.001
Dragons in Danger (2020)	-6.799	<0.001	-4.408	<0.001
Sumatra (2020)	-3.633	<0.001	-6.352	<0.001

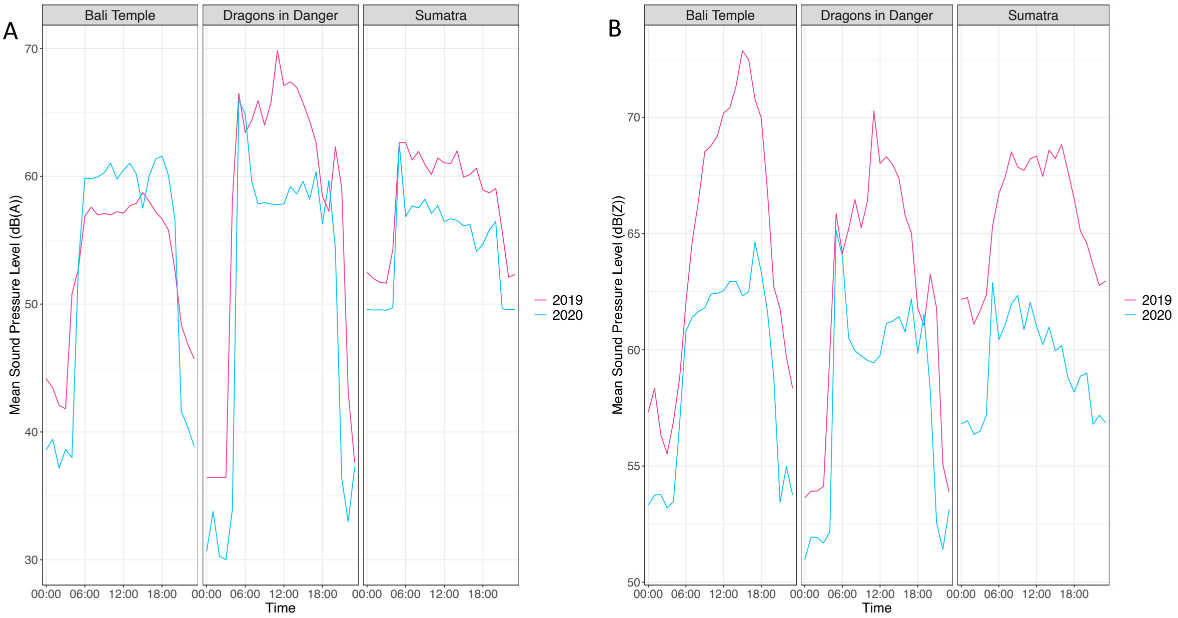

Figure 7.1: Daily patterns of sound pressure levels (wide frequency band) for zoo closure (2020) and zoo open (2019) periods across three zoo aviaries (Bali Temple, Dragons in Danger and Sumatra). Panel A shows the mean sound pressure levels in dB(A). Panel B shows mean sound pressure levels in dB(Z).

7.5.2 Defined frequency band measures

When considering sound pressure levels within frequency bands, changes in sound pressure levels between years varied among aviaries and frequency bands. As the models contained significant interactions, we present pairwise differences for frequency band and year to show how sound within each band changed between the zoo open and zoo closed periods. In the Bali Temple aviary, both very low and low frequency sound decreased in 2020 compared to 2019 (Table 7.3; Figure 7.2) However, mid frequency sound did not change between the two years, and High frequency sound increased in 2020 compared to 2019 (Table 7.3; Figure 7.2). In both the Dragons in Danger and Sumatra aviaries mean sound pressure levels decreased in 2020 for all frequency bands (Table 7.3; Figure 7.2). Across all models, the spline for time was significant ($p<0.001$), meaning that sound pressure levels varied significantly over the course of the day.

Table 7.3: Results from Generalized Additive Models (GAMs) examining the effect of year and frequency band (Very Low (17.6-111.4 Hz), Low (111.4 – 890.9 Hz), Mid (890-9 – 8979.7 Hz), High (8979.7 – 11313.7 Hz)) on sound pressure levels (dB(Z)) in three Chester Zoo aviaries (Bali Temple, Dragons in Danger and Sumatra). Values represent pairwise differences illustrating the change in mean sound pressure level (SPL) within each frequency band during zoo closure in 2020 compared to zoo opening in 2019

	Estimate	p-value
Bali Temple		
Very Low (2020)	-7.704	<0.001
Low (2020)	-7.043	<0.001
Mid (2020)	0.201	0.783
High (2020)	4.391	<0.001
Dragons in Danger		
Very Low (2020)	-2.607	0.002
Low (2020)	-9.745	<0.001
Mid (2020)	-5.891	<0.001
High (2020)	-3.168	<0.001
Sumatra		
Very Low (2020)	-8.001	<0.001
Low (2020)	-4.331	<0.001
Mid (2020)	-3.800	<0.001
High (2020)	-2.140	<0.001

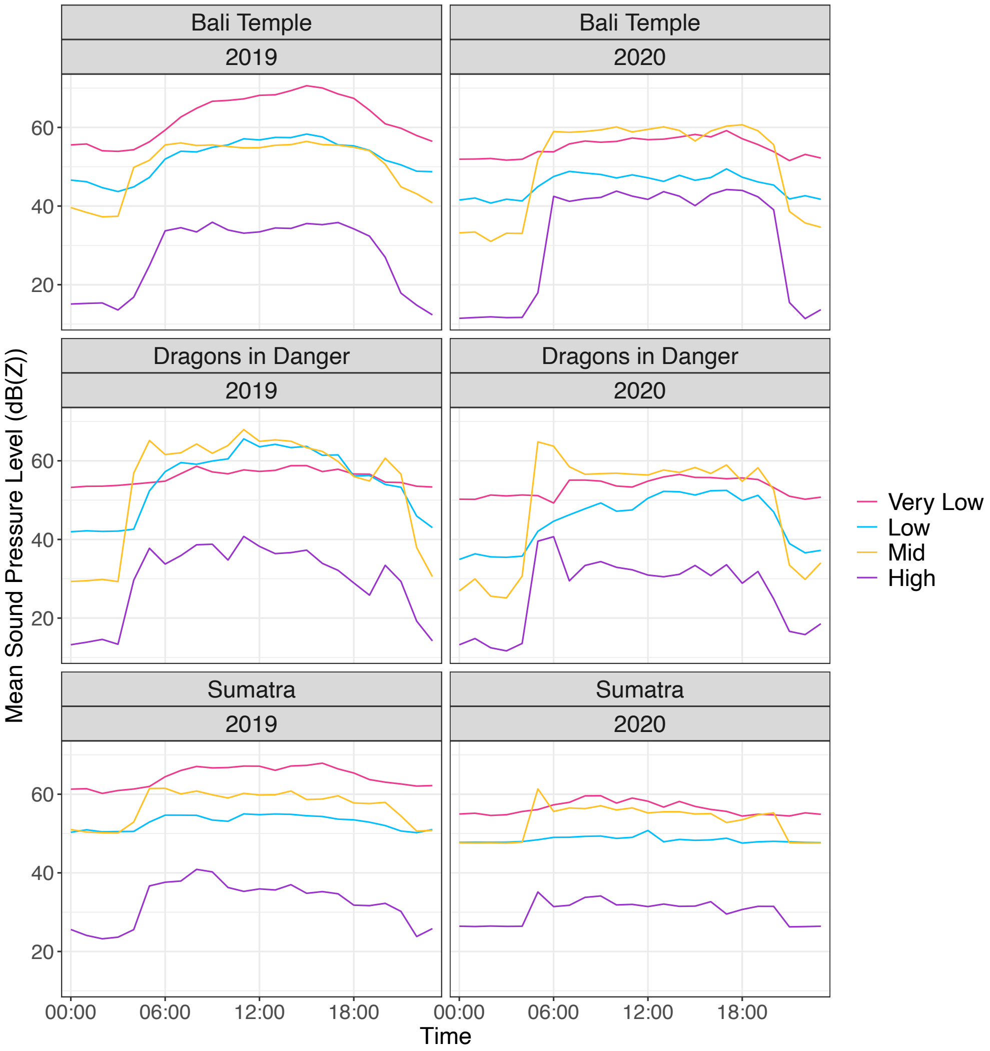

Figure 7.2: Mean sound pressure levels (dB(Z)) within frequency bands (Very Low (17.6-111.4 Hz), Low (111.4 – 890.9 Hz), Mid (890-9 – 8979.7 Hz), High (8979.7 – 11313.7 Hz)) for zoo closure (2020) and zoo open (2019) periods in three zoo aviaries (Bali Temple, Dragons in Danger and Sumatra).

7.5.3 Acoustic Indices

There was a significant effect of year on the Acoustic Complexity Index (ACI), although the direction of this effect differed between aviaries (Table 7.4; Figure 7.3A). In the Bali Temple aviary, ACI values were higher in 2020 than 2019, indicating a more complex soundscape. However, in both the Dragons in Danger and Sumatra aviaries, ACI values were lower in 2020 than 2019, indicating reduced soundscape complexity in 2020. Across all models, the spline for time was significant ($p<0.001$), meaning that sound pressure levels varied significantly over the course of the day.

Table 7.4: Generalized Additive Models (GAMs) examining the effect of year (as a factor) on Acoustic Complexity Index (ACI) and Normalized Difference Soundscape Index (NDSI) in three zoo aviaries (Bali Temple, Dragons in Danger and Sumatra). Estimates represent the difference in 2020 compared to 2019.

	ACI		NDSI	
	Estimate	p-value	Estimate	p-value
Bali Temple (2020)	5.549	<0.001	0.252	<0.001
Dragons in Danger (2020)	-3.478	0.006	0.459	<0.001
Sumatra Aviary (2020)	-8.475	<0.001	-0.032	0.317

When considering the Normalized Difference Soundscape Index (NDSI), there was a significant effect of year in two out of the three aviaries examined. In both the Bali Temple and Dragons in Danger aviaries, NDSI values were higher in 2020 than 2019 (Table 7.4; Figure 7.3B). This indicates a greater proportion of biotic compared to anthropogenic sound in the soundscape. However, there was no significant difference in NDSI values between years in the Sumatra aviary (Table 7.4; Figure 7.3B). Across all models, the spline for time was significant (p<0.001).

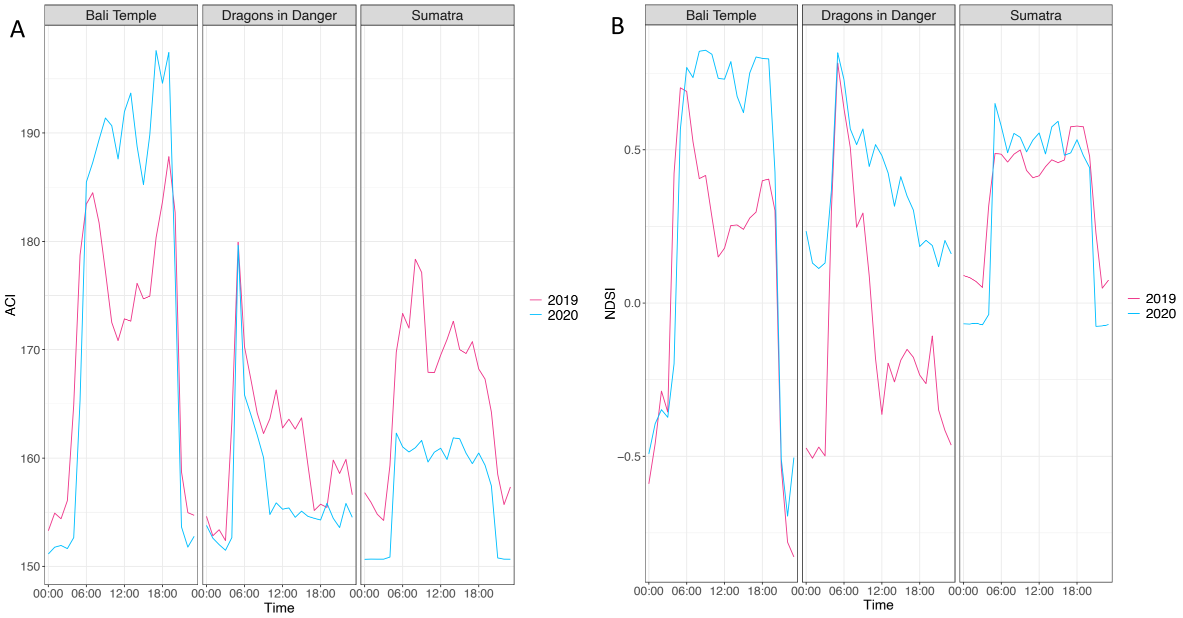

Figure 7.3: Daily patterns of acoustic indices in zoo aviaries for zoo closure (2020) and zoo open (2019) periods in three zoo aviaries (Bali Temple, Dragons in Danger and Sumatra). Panel A shows the Acoustic Complexity Index (ACI). Panel B shows the Normalized Difference Soundscape Index (NDSI).

7.6 Discussion

We found significant differences in the sound environment of zoo aviaries between the COVID-19 lockdown period of zoo closure in 2020 and a similar period of normal zoo operation in 2019. The COVID-19 lockdown period provided a unique opportunity to explore the effects of human activity on animals (Rutz et al. 2020), and this was also true in zoos. The zoo remained closed to visitors for the duration of the lockdown, meaning that sound associated with visitors was not present during 2020. This includes visitor speech, sound associated with footfall, and the use of enclosure furnishings, such as doors. As well as a reduction in the number of people within the zoo, the lockdown is also likely to have reduced human activity surrounding the zoo due to reductions in human movement. As such, volume of traffic from cars and other transport around the zoo would have been reduced, resulting in fewer sources of anthropogenic sound. The lockdown is unlikely to have significantly affected sound related to daily zoo husbandry and management. Enclosure machinery, such as HVAC (heating, ventilation and air conditioning) systems, would still have been functioning during the closure, although these may have performed more efficiently in the absence of visitors e.g., due to doors opening fewer times. Similarly, animal care and enclosure servicing were still ongoing throughout the lockdown. Given the substantial reductions in human activity during 2020, it is apparent that chronic human presence in and around the zoo has a significant effect on the zoo soundscape. In general, aviaries had lower sound pressure levels during zoo closure for wide band frequency measures and within narrow frequency bands. However, these effects differed between aviaries and frequency bands, with some showing no difference between years and others increasing during the zoo closure period. Soundscape complexity did not show a consistent pattern between the two years. However, soundscape naturalness was generally higher during zoo closure, indicating a greater proportion of biotic compared to anthropogenic sound in 2020.

7.6.1 Wide frequency and defined frequency band measures

Overall sound pressure levels across wide frequency measures decreased during the zoo closure period. We found similar, but not identical, results when considering A-weighted (dB(A)) and Z-weighted (dB(Z)) metrics. When considering dB(A), sound pressure levels decreased in two of the three aviaries, and remained unchanged in the third. However, for dB(Z), sound pressure levels decreased in all three aviaries and patterns were more pronounced. This pattern is similar to that reported by Quadros et al., (2014), who found that sound levels were lower on days when the zoo was open compared to when the zoo was closed across enclosures measured, although only the A-weighted metric was considered. Differences between results using dB(A) and dB(Z) in this study may relate to the presence of lower frequency sound in the environment. These lower frequencies are down-weighted when calculating dB(A), which is specifically weighted to reflect human hearing sensitivity (Kurra 2021). In line with this, we found that low frequency sound (very low (17.6 –

111.4 Hz) and low (111.4 – 890.9 Hz) frequency bands) was a significant component of the sound environment in all aviaries and decreased during the zoo closure period. However, changes for mid (890.9 – 8979.7 Hz) and high (8979.7 – 11313.7 Hz) were not consistent across aviaries. Although sound pressure levels in these bands decreased during the zoo closure period in two of the aviaries, in the third, there was no change in the mid frequency band and increased the high frequency band. The mid and high frequency bands are likely to have substantial contributions from biotic sound, so this may indicate that biotic sound responded differently to zoo closure among aviaries, with higher sound pressure levels indicating more biotic sound during closure. Our results indicate that dB(A) scales may be less useful when examining anthropogenic sound in the environment. In addition, whilst dB(A) may be appropriate for animals with hearing ranges similar to humans, it is inappropriate for species with different hearing ranges (Pater et al. 2009). The use of an unweighted metric, such as dB(Z), may be more useful when characterizing anthropogenic impacts on the sound environment, and the use of narrow frequency bands can help to more accurately pinpoint where these impacts occur and what species they are most likely to affect.

The sound environment in different aviaries was differentially affected by reduced human presence during the period of zoo closure. These differences could relate to a number of factors, including aviary position within the zoo, popularity with visitors, aviary setting (indoor or outdoor), and species composition. Pelletier et al. (2020) found differences between indoor and outdoor environments, with indoor environments being significantly louder. However, we did not find that the sound environment in the indoor aviary in this study, Dragons in Danger, responded differently to zoo closure than the outdoor aviaries. Instead, the Sumatra and Dragons in Danger aviaries showed similar responses for all metrics. The Bali Temple aviary, on the other hand, differed in several ways, with no difference in dB(A) between years and different patterns with respect to changes within frequency bands. It is possible that the location of this aviary near to the zoo perimeter affected the sound environment due to the proximity to outside roads. The number of birds in this enclosure also increased between 2019 and 2020, which may have contributed to different patterns in higher frequency bands representing biotic sound. Aviaries also differed in temporal patterns. All aviaries had increased sound pressure levels early in the morning, likely corresponding with sunrise and the dawn chorus. The characteristics of this increase differed between the indoor and outdoor aviaries. Outdoor aviaries had a more gradual increase in sound levels that differed between years and aviaries. Some temporal variation is likely caused by differing sunrise times during different recording periods, and a more gradual increase in light intensity. Outdoor aviaries are also influenced by native birds, which may behave differently to zoo birds. In the indoor aviary, there appeared to be a large increase in sound level at around 05:00, which corresponds to the time when the enclosure lighting comes on and subsequent dawn chorus The early morning peak is most clear in 2020, which is likely because there is no visitor sound during the day to sustain sound levels. Given the number of potential factors involved, examining a wider range of enclosures is necessary to determine which factors are most important in determining the sound environment and changes therein.

The effect of human presence on environmental sound in zoos has a range of implications for animals and their management. The effects of increased environmental sound on animals have been well studied (reviewed in Barber et al., 2010; Blickley & Patricelli, 2010; Kight & Swaddle, 2011). Sound exposure can influence animal welfare, resulting in altered behaviours, changes in or loss of hearing, and altered physiology (Kight & Swaddle 2011). Animal welfare is a key component of modern zoos and aquaria, with collections striving for high standards of care (Mellor et al. 2005). However, despite its importance, the auditory environment remains one of the least studied aspects of animal welfare (Binding et al. 2020). Increased sound can also influence the reproductive behaviour of animals, reducing success, which could negatively impact ex situ breeding programmes. In great tits (*Parus major*) females laid smaller clutches in noisier areas (Halfwerk et al. 2011). Similar decreases in reproductive success were also reported in eastern bluebirds (*Sialia sialis*) (Kight et al. 2012). Given the potential effects on welfare, zoos may wish to use mitigation measures to reduce the impact of sound on animals. Sound absorbing barriers were successful in reducing sound produced by an HVAC system, although different materials had different levels of effectiveness and this varied between frequency bands (Orban et al. 2017). Therefore, an understanding of sound pressure levels in narrower frequency bands may be more beneficial than wide-band measures for determining the effectiveness of such interventions.

Increases in environmental sound may also result in masking of important sounds relating to survival and reproduction (Brumm & Slabbekoorn 2005; Barber et al. 2010; Blickley & Patricelli 2010). Masking of adventitious sounds, such as those related to movement of predators (Barber et al. 2010), is likely to be less important in captive environments, as animals will not encounter threats in the same was as animals in situ. However, human influences on environmental sound have the potential to influence vocal behaviour of birds and other animals in zoos. Birds in urban environments often sing louder or use higher frequencies than those in more rural environments, which may be interpreted as an adaptation to mitigate masking by low frequency anthropogenic sound (Slabbekoorn et al. 2003; Luther & Baptista 2009; Nemeth & Brumm 2009). Changes in soundscape due to the COVID-19 lockdowns significantly affected the vocal behaviour of white-crowned sparrows (*Zonotrichia leucophyrs*) (Derryberry et al. 2020). Birds in urban areas sang more quietly and exhibited greater vocal performance by increasing frequency bandwidth of songs in response to release from masking by low-frequency sound. Similar changes in captive environments could contribute to vocal change and divergence during ex situ management. Vocal divergence could negatively impact conservation programmes by affecting mate preference and assortative mating (Lewis et al. 2021). For example, in golden mantella frogs (*Mantella aurantiaca*), captive and wild frogs had significantly different vocalizations, and captive frogs responded more strongly to calls from their own population than the wild population (Passos et al.

2017). Examining the vocal behaviour of individual species in response to the zoo soundscape could, therefore, be useful in establishing how human presence in zoos influences vocal behaviour in zoo birds to determine the potential role in conservation programmes.

7.6.2 Acoustic indices

We also examined the potential for using acoustic indices to describe the soundscape in zoos. Acoustic indices are frequently applied to the study of soundscapes in wild environments (Sueur et al. 2014), but are not generally considered in captive environments (Clark & Dunn 2022). The use of acoustic indices can provide us with information about the characteristics of sound in the environment beyond its intensity (Sueur et al. 2014) and allow for investigation and comparison of the soundscape between enclosures, zoos, and environments.

The Acoustic Complexity Index (ACI) is a measure of soundscape complexity, examining variation in amplitude between adjacent time points within narrow frequency ranges (Pieretti et al. 2011). In situ, ACI correlates with the number of avian vocalizations (Pieretti et al. 2011), suggesting that this index could be used as a proxy for vocal activity in captive and other environments. Vocal activity can be a useful measure for husbandry and management. Many birds increase calling activity near to the breeding season (Amrhein et al. 2002; Koloff & Mennill 2013; Digby et al. 2014), so increases in activity could be used to determine timing of management actions such as nest box provision. Without the use of an index, this would require subjective perception of calling activity or require counting of calls live within the aviary or from a recording, which may be prohibitively time consuming. Changes in vocal activity could also be used to assess disturbances or interventions; for example, peaks of vocal activity may change based on levels of anthropogenic sound disturbance (Brumm & Slabbekoorn 2005; Slabbekoorn & Ripmeester 2008; Ortega 2012). Although ACI and vocal activity could be useful in informing zoo management, we did not find consistent patterns among aviaries. Complexity was higher in the Bali Temple aviary in 2020 compared to 2019, but lower in both the Dragons in Danger and Sumatra aviaries. However, ACI values showed expected patterns relating to time of day, increasing during daylight hours when birds are likely to be active, suggesting it could provide useful information. A number of factors could have influenced our results. Most importantly in captive environments, human vocalizations are likely to contributed to soundscape complexity, which may have contributed to increased sound complexity during zoo opening in some aviaries. In addition, vocal activity in birds is affected by a range of different factors including the time of year (Amrhein et al. 2002; Koloff & Mennill 2013; Digby et al. 2014), and weather (O'Connor & Hicks 1980; Digby et al. 2014; Vokurková et al. 2018). Differences in these parameters between years may have affected vocal activity in the two periods, contributing to differences in vocal activity and thus complexity. Changes in aviary composition between the two years could also have contributed to the observed patterns; the number of birds increased in the Bali temple between 2019 and 2020, but remained relatively consistent in the Sumatra aviary and decreased in Dragons in Danger. Differences in the number of birds is likely to affect the overall number of vocalizations, potentially contributing to changes in ACI. Given the range of factors that could influence complexity, further investigation is required to fully understand possible factors that influence the ACI in zoos and the relevance of soundscape complexity in captive environments.

The Normalized Difference Soundscape Index (NDSI) is a measure of 'naturalness' which compares the relative contribution of anthropogenic and biotic sound to the soundscape (Kasten et al. 2012). NDSI values were higher, indicating a greater proportion of biotic sound and therefore relatively less anthropogenic sound, in 2020 for both the Bali Temple and Dragons in Danger aviaries, although values were similar in both years in the Sumatra aviary. As with ACI, NDSI showed similar temporal patterns to sound pressure levels, increasing during daylight hours when birds were more active and biotic sound is expected to increase. Captive environments generally have higher levels of anthropogenic sound than those in situ (e.g., Lara & Vasconcelos, 2019). Soundscape naturalness, and the NDSI, may, therefore, be a useful measure to determine the impact of anthropogenic sound sources on the sound environment in enclosures and to determine if mitigations are required. The NDSI could also be used to compare the sound environment between wild and captive settings, as well as among collections and enclosures. Not only could this help with zoo management by identifying locations where sound mitigation may be required, it could also identify areas with relatively little anthropogenic disturbance that could be used to house sensitive species. Although the NDSI appears to be a useful measure in zoos, there are a number of important considerations when using this index. Firstly, the standard settings for the index do not consider sounds below 1000 Hz. However, from our analysis of sound pressure levels within frequency bands, it is clear that frequencies below 1000 Hz are a substantial component of the sound environment. As NDSI values are calculated by comparing sound in low and high frequency bands (Kasten et al. 2012), it may not be suitable for use in all enclosures. Animals with low frequency vocalizations that appear in the 'anthropogenic' frequency range, i.e., between 1 and 2 kHz, will affect the performance of the index. For example, the presence of the common loon (*Gavia immer*) resulted in low, negative NDSI values due to its low frequency call (Kasten et al. 2012). Species in captive environments with similar vocal ranges may have a similar effect on NDSI values. Whilst this does not preclude the use of NDSI, it is important to consider how species composition could affect the index and the relevance of this for the research question. Perhaps the most important consideration for using the NDSI in zoos is how sound from visitors influences the index. Human speech covers a broad range of frequencies, with fundamental frequencies below the ranges specified by the NDSI (~60 – 200 Hz in adult males) (Fant 2004). In adult males, the range of the first four formants of speech (F1-F4) spans ~ 180 to 4000 Hz, and female voices, on average, have higher formant frequencies (Fant 2004). As such, human voices span both the ‘anthropogenic’ and ‘biotic’ bands of the NDSI. Whilst we were still able to detect differences between the time periods, a better understanding of how human speech influences the NDSI would be beneficial to fully comprehend its role for monitoring sound in zoos.

7.6.3 Conclusions and recommendations

Understanding the zoo soundscape is important for zoo management. For example, sensitive species may be more suited to quieter areas away from sound sources, such as external roads, visitor attractions (Pelletier et al. 2020), or construction (Jakob-Hoff et al., 2019; Williams et al., 2021). When incorporating the sound environment into management plans, it is also necessary to consider species’ sensitive hearing ranges, as sounds within this range will have a greater impact than those outside of it. The frequencies of sound examined in this species were appropriate for birds (Dooling 1992; Dooling et al. 2000; Catchpole & Slater 2008). However, many animals are sensitive to frequencies outside of this range, such as ultrasound (e.g., rodents (Sales 2010)), and this should be considered where relevant.

Based on our findings, we propose recommendations for the study of the zoo sound environment. Examining multiple facets of the sound environment, including sound levels and acoustic indices, may help to provide a more holistic picture of sound in zoos and help us separate the sound environment from visitor presence. We found that acoustic indices, particularly NDSI, have potentially useful applications in captive settings. However, further examination into the use of these acoustic indices in captive environments is necessary, including how factors such as human speech affect results. In addition, there are a wide range of other indices available that may prove useful in zoos. Finally, we suggest that examining how the soundscape influences animal behaviour, and in particular vocal behaviour, is an important avenue for further research.

7.7 Author Contributions

RL and LW conceptualized the study, with all authors contributing to refining data extraction protocols. RL and LW were involved in data collection for the project. RL completed the extraction of sound measures. RL and TG were responsible for data analysis. RL wrote the initial draft of the manuscript, including visualizations, with all authors contributing to editing the manuscript. LW, SdK, SS, and TG provided supervision to RL throughout the course of the project.

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7.10 Supplementary Information

Supplementary Information 7.1: Species lists for the three zoo aviaries in which the sound environment was investigated (Bali Temple, Dragons in Danger, Sumatra Aviary) in 2019 during zoo opening and 2020 during zoo closure

Bali Temple

2019			***2020***		
Species Name	***Latin Name***	***Number***	***Species Name***	***Latin Name***	***Number***
Bali myna	*Leucopsar rothschildi*	9	Bali myna	*Leucopsar rothschildi*	5
Java sparrow	*Lonchura oryzivora*	55	Java sparrow	*Lonchura oryzivora*	65
Pied imperial pigeon	*Ducula bicolor*	5	Magpie robin	*Copsychus saularis*	2
Purple-naped lory	*Lorius domicella*	2	Pied imperial pigeon	*Ducula bicolor*	5
Sumatran laughing thrush	*Garrulax bicolor*	1	Purple-naped lory	*Lorius domicella*	4
Yellow-backed chattering lory	*Loris garrulus flavopalliatus*	1	Sumatran laughing thrush	*Garrulax bicolor*	1
			Yellow-backed chattering lory	*Loris garrulus flavopalliatus*	1

Sumatra aviary

2019			***2020***		
Species Name	***Latin Name***	***Number***	***Species Name***	***Latin Name***	***Number***
Asian glossy starling	*Aplonis panayensis*	24	Asian glossy starling	*Aplonis panayensis*	26
Bronze-tailed peacock pheasant	*Polyplectron chalcurum*	2	Bronze-tailed peacock pheasant	*Polyplectron chalcurum*	2
Chestnut-backed thrush	*Geokichla dohertyi*	4	Chestnut-backed thrush	*Geokichla dohertyi*	5
Chestnut-bellied tree partridge	*Arborophila javanica*	1	Chestnut-bellied tree partridge	*Arborophila javanica*	1

Emerald dove	*Chalcophaps indica*	9	Emerald dove	*Chalcophaps indica*	6
Fairy bluebird	*Irena puella*	1	Fairy bluebird	*Irena puella*	1
Fire-tufted barbet	*Psilopogon pyrolophus*	1	Fire-tufted barbet	*Psilopogon pyrolophus*	1
Javan green magpie	*Cissa thalassina*	3	Javan green magpie	*Cissa thalassina*	2
Magpie robin	*Copsychus saularis*	1	Salvadori's pheasant	*Lophura inomata*	2
Salvadori's pheasant	*Lophura inomata*	2	Silver-eared mesia	*Leiothrix argentauris*	7
Silver-eared mesia	*Leiothrix argentauris*	3			

Dragons in Danger

2019			***2020***		
Species Name	***Latin Name***	***Number***	***Species Name***	***Latin Name***	***Number***
Black-naped fruit dove	*Ptiliopus melanospilus*	3	Black-naped fruit dove	*Ptiliopus melanospilus*	4
Cinnamon ground dove	*Gallicolumba rufigula*	4	Cinnamon ground dove	*Gallicolumba rufigula*	3
Fairy bluebird	*Irena puella*	3	Fairy bluebird	*Irena puella*	2
Great argus	*Argusianus argus*	2	Great argus	*Argusianus argus*	2
Luzon bleeding heart dove	*Gallicolumba luzonica*	6	Javan green magpie	*Cissa thalassina*	2
Malayan great argus	*Argusianus argus argus*	1	Luzon bleeding heart dove	*Gallicolumba luzonica*	6
Mindanao bleeding heart dove	*Gallicolumba criniger*	2	Malayan great argus	*Argusianus argus argus*	1
Montserrat oriole	*Icterus oberi*	2	Mindanao bleeding heart dove	*Gallicolumba criniger*	3
Palawan peacock pheasant	*Polyplectron superbus*	2	Palawan peacock pheasant	*Polyplectron superbus*	1
Philippine mouse-deer	*Tragulus nigricans*	1	Philippine mouse-deer	*Tragulus nigricans*	1
Pink-headed fruit dove	*Ptilinopus porphyrea*	1	Pink-headed fruit dove	*Ptilinopus porphyrea*	1
Sumatran laughing thrush	*Garrulax bicolor*	2	Superb fruit dove	*Ptilinopus superbus*	5
Superb fruit dove	*Ptilinopus superbus*	4	Victoria crowned pigeon	*Goura victoria*	1
Visayan tarictic hornbill	*Penelopides panini panini*	5	Visayan tarictic hornbill	*Penelopides panini panini*	2
White-naped pheasant pigeon	*Otidiphaps aruensis*	2	White-naped pheasant pigeon	*Otidiphaps aruensis*	2

8. General discussion

Ex situ breeding and management are important tools in conservation programmes. However, adaptation to captivity and behavioural change during ex situ management may reduce the success of conservation interventions involving captive populations. Therefore, understanding these processes is a key step to improving the efficacy of conservation efforts. This thesis focuses on two key questions; firstly, how can mating traits, specifically vocal behaviour, change during ex situ breeding programmes; and secondly, how could these changes affect conservation efforts. Firstly, I aimed to understand the key roles that vocal behaviour may play in influencing conservation programmes. Then, I explored vocal behaviour and its possible roles in the conservation of the Java sparrow (*Lonchura oryzivora*), an endangered estrildid finch. The Java sparrow is a useful model species for a number of reasons: 1) the Java sparrow is an endangered species of conservation concern (Lee et al. 2016; BirdLife International 2018); 2) the species is frequently found in ex situ populations, meaning it is easily accessible and has significant potential for vocal change during captive breeding; 3) vocal behaviour is well studied in some of its close taxonomic relatives, the zebra finch (*Taeniopygia guttata*) and the Bengalese finch (*Lonchura striata domestica*); and 4) Java sparrows have a simple vocal repertoire of a single song type with up to 8-10 note types (Ota & Soma 2014) meaning patterns associated with vocal behaviour can be studied more easily in this species than in those with more complex vocal behaviour.

Chapter 2 examines the role of vocal behaviour in conservation programmes. In some cases, vocal behaviour can be beneficial to conservation efforts, with uses in population surveys and monitoring. However, I also identify a range of possible negative effects. In particular, there is a significant role of vocal learning and the formation of vocal dialects. Preferences for familiar dialects may contribute to assortative mating during conservation programmes, limiting the success of interventions.

Given the potential importance of vocal learning in conservation programmes, in Chapters 3 and 4, I investigate song inheritance in the Java sparrow to determine how different modes of inheritance influence song phenotype. I find that the majority of song features, including song structure and complexity, the acoustic structure of notes, and temporal features of song are socially learned, with son's songs resembling those of their social father. As such, there is the potential for cultural change within populations, resulting in vocal divergence. In Chapter 5, I explored how preference for familiar songs might influence mating behaviours in Java sparrows, finding that females showed significant preferences for familiar, rather than unfamiliar songs. As identified in Chapter 2, preferences for familiar songs may result in assortative mating based on familiarity, hindering conservation efforts.

As well as vocal behaviour itself, I also examined other mating traits and potential drivers of vocal change in captive environments. In Chapter 6, I examine morphological differences between wild and captive populations of Java sparrows. Not only is morphology an important trait in its own right, with implications for fitness of released individuals, but it can also influence song phenotype. Birds in captive environments were significantly larger and had more pronounced sexual size dimorphism than wild type birds. Changes in morphology may influence the performance of captive individuals in release programmes and have the potential to reduce the fitness of wild populations during supportive breeding. Finally, in Chapter 7, I explored the sound environment in zoological collections, which could be a driver of vocal change in captive environments, as is the case in urban environments. I find a distinct influence of human presence on the sound environment in the zoo, with significant differences between normal zoo opening and zoo closure during the COVID-19 lockdown period. Specifically, aviaries in the zoo were louder with comparatively more anthropogenic noise during normal opening than during closures.

Taken together, this body of work demonstrates that there is the potential for vocal divergence during ex situ breeding of bird populations, and that this divergence can have knock-on effects for reproductive behaviour. In the rest of this section, I discuss the consequences of these findings for conservation efforts, and more specifically ex situ breeding programmes, and the potential management implications of changes in vocal behaviour during ex situ breeding.

8.1 What influences vocal change in captive breeding?

In Chapter 2, I identified a range of situations where vocal behaviour could influence conservation interventions. These were not limited to ex situ breeding programmes, but many of the same principles, such as the importance of vocal learning, have applications in captive management. In this thesis, these principles were further explored in relation to ex situ breeding programmes, exploring song inheritance, correlations with other traits, and environmental differences. Whilst this thesis focused on the Java sparrow as a model species, several broad trends were apparent which could be applied to ex situ management across species, especially for those that learn their vocalizations.

8.1.1 Management of small populations

Conservation programmes, including ex situ breeding programmes, are often characterized by the management of small, isolated populations (WAZA 2005). This small population management during ex situ breeding has the potential to influence vocal behaviour in many species. In Chapter 2, I identified vocal learning as a key factor that could influence the success of conservation interventions through vocal divergence and the formation of population dialects. In Chapters 3 and 4, it was demonstrated that social learning plays an important role in the inheritance of vocal behaviour in the Java sparrow, and that this outweighs contributions from genetic factors. As such, vocalizations in this, and other species that learn their vocalizations are likely to be affected by cultural processes, such as cultural drift and bottlenecks.

Cultural drift describes the random accumulation of mutations in songs due to copying errors and improvisation (Mundinger 1980), and this can lead to population-specific variation in both space (e.g., Koetz et al., 2007) and time (e.g., Nelson et al., 2004). As with genetic drift, cultural drift is expected to act more strongly in small, fragmented populations (Laiolo & Tella 2007), like those involved in ex situ breeding programmes. Furthermore, population isolation is an important driver of sound variation, acting in tandem with cultural drift, to allow for the build-up of mistakes or innovations between populations (Laiolo 2010). In situ, isolation may result from geographic barriers (e.g., Ortiz-Ramírez et al. 2016; Purushotham & Robin 2016). In ex situ populations, 'barriers' between populations may be a result of limited movement between populations housed in different collections, increasing the likelihood for vocal divergence. As such, management of species in small, isolated populations can result in vocal divergence, even in the absence of other factors.

Reduction in population sizes, e.g., due to habitat loss or colonization of new areas, can result in cultural bottlenecks, which can contribute to the loss of acoustic diversity in species that learn vocalizations (Baker & Jenkins 1987; Baker 1996; Laiolo & Tella 2007; Laiolo 2010; Hill et al. 2013; Lachlan et al. 2013). These bottlenecks can also be introduced during conservation programmes, such as when small groups of animals are moved for conservation purposes (Lewis et al. 2021). For example, serial translocations of North Island saddlebacks (*Philesturnus rufaster*) resulted in reduced levels of song type sharing with the ancestral population, with larger effects following multiple interventions (Parker et al. 2012). In ex situ breeding programmes, bottlenecks may occur when small groups of animals are moved to new collections or locations. The continued action of drift following bottlenecks can result in significant differences in vocal behaviour between populations despite similar levels of song diversity (Lang & Barlow 1997; Hamao & Ueda 2000; Lachlan et al. 2013). In this way, movement of birds and the formation of new populations during ex situ breeding programmes may encourage vocal divergence between populations.

8.1.2 Reduced learning opportunities

In species where vocal learning is important in the development of adult song phenotype, reduced opportunities to learn may affect vocal development in captivity. In a range of species, birds raised without a tutor can develop atypical vocalizations, with unusual note structures, decreased stereotypies, and temporal abnormalities (Price 1979; Marler & Sherman 1985; Chaiken et al. 1993; Feher et al. 2009; Kagawa et al. 2014). In an analysis of regent honeyeater (*Anthochaera phrygia*) songs, songs of individuals that had been raised in captivity had lower complexity than birds of wild origin (Crates et al. 2021). Captive juveniles are typically housed away from adults, so do not have the opportunity to learn from adult conspecifics, potentially contributing to different song cultures in captivity (Crates et al. 2021).

Birds tutored by heterospecifics often incorporate song features from the tutor species into their vocalizations, rather than solely producing species-typical songs (Johannessen et al. 2006; Eriksen et al. 2009; Mann et al. 2021). In addition, where learning opportunities from conspecifics are limited, species may incorporate information from other species into their vocal phenotype. Interspecific singing, where individuals sing the songs of other species, in the regent honeyeater was associated with population density; interspecific singers had significantly fewer conspecifics in the surrounding area (Crates et al. 2021). As such, reduced learning opportunities during ex situ breeding could result in maladaptive song learning and reduced fitness.

Reduced learning opportunities could influence vocal behaviour during the formation of new populations. The withdrawal of learning theory (Thielcke 1973) predicts rapid innovation following colonization of new habitats. If founders were young birds that dispersed before song crystallization, large radiations of new syllables could appear during colonization due to a lack of model songs. For example, in silvereyes (*Zosterops lateralis*), newly colonized populations did not show reduced syllable diversity compared to older populations (Potvin & Clegg 2015). This does not support gradual drift following colonization, as mentioned in 8.1.1, but rather a radiation of syllables due to withdrawal of learning or release from selection post-colonization.

A lack of appropriate stimuli in the captive environment may also affect vocal behaviour. Captive Hawaiian crows (alala, *Corvus hawaiiensis*) produced fewer alarm calls and lacked the territorial broadcast calls recorded in past wild populations (Tanimoto et al. 2017). One factor affecting call production may be a reduced need to use these calls in the captive environment. This, in turn, may affect cultural transmission, as vocalizations become less common due to changes in social and physical settings. Direct experience can be important in shaping vocalizations relevant for survival, such as alarm calls (Hollén & Radford 2009). In addition to affecting vocal behaviour, a lack of appropriate stimuli in the captive environment may influence animals’ responses to auditory stimuli (e.g., Friant et al., 2008; Sabol et al., 2022), the implications of which are discussed further in 8.2.2.

I did not explore the role of reduced learning opportunities in the development of adult song phenotype in Java sparrows. However, given the important role of social learning for song inheritance, as demonstrated in Chapters 3 and 4, limited access to tutors is likely to negatively affect song development. As such, avoiding reductions in learning opportunities is likely to be beneficial in conservation programmes for this and other species.

8.1.3 Differing conditions and changes in correlated traits during ex situ management

Many species undergo a suite of interlinked phenotypic and genetic changes during ex situ management (Frankham 2008; Williams & Hoffman 2009; Schulte-Hostedde & Mastromonaco 2015). These often result from necessary differences between wild and captive environments, such as dietary changes or removal of predators (Frankham 2008). Differences in conditions and selection pressures during ex situ management could affect vocal behaviour in birds.

Dietary differences between wild and captive environments could contribute to changes in vocal behaviour. Young birds are susceptible to nutritional stress during early development, which can result in a trade-off between investment in the song system and other features (Nowicki et al. 1998, 2002a; Nowicki & Searcy 2005). This can, therefore, affect song phenotype (Nowicki et al. 2002a; Zann & Cash 2008; Brumm et al. 2009). In zebra finches, nutritional stress was associated with reduced song complexity (Zann & Cash 2008), suggesting that dietary differences could also contribute to vocal differences between populations. In swamp sparrows (*Melospiza georgiana*), poor early nutrition was associated with less accurate copying of song models and a significant reduction in the size of song system nuclei (Nowicki et al. 2002a). If changes in early nutrition affect copy accuracy, dietary differences between populations could contribute to vocal divergence through cultural processes. As well as dietary differences, parasite infection early in life can affect adult song phenotype (Spencer et al. 2005). Parasite loads are likely to be reduced in captive populations compared to those in the wild (Frankham 2008), which could contribute to changes in vocal behaviour. In Chapters 3 and 4, I did not find a significant effect of the developmental environment on a number of song features, including song structure, note acoustic structure, and temporal features, although some measures of vocal consistency were influenced by developmental environment. In a single captive population, as examined in these studies, there is unlikely to be high levels of variation in the developmental environment among nests, especially with respect to diet and parasite loads. However, differences in features of the developmental environment between wild and captive environments, and even between captive environments, are likely to be more pronounced, so diet and the developmental environment may still be important factors contributing to vocal divergence in captive populations.

Changes in correlated traits, such as morphology, can have knock-on effects for vocalizations. Morphology and vocal behaviour are linked in many species, with both overall body size and beak morphology influencing vocalizations (Podos 2001; Huber & Podos 2006; Derryberry et al. 2012, 2018; García & Tubaro 2018). In Chapter 6, I examined morphological differences between captive populations and wild museum specimens, finding that birds from both zoos and aviculture were larger and had more pronounced sexual size dimorphism across most of the measures examined.

In Java sparrows, trill performance, a statistic which reflects average trill rate and frequency bandwidth of trills, is associated with body size, with larger birds producing higher performance trills (Kagawa & Soma 2013). As such, morphological differentiation in captive populations could contribute to vocal differentiation between populations. Changes in morphology could be a plastic response to changed environments (e.g., diet (Boag 1987; Johnston 1993), parasite load (Potti & Merino 1996; Fessl et al. 2006)), however, there is also likely to be an effect of inadvertent selection during ex situ breeding programmes (Champagnon et al. 2010; Chargé et al. 2014). The precise mechanism for morphological differences between wild and captive populations of Java sparrows was not explored. However, it is expected that overall morphological change is likely to be a combination of plasticity and selection.

The effects of changing selection pressures during captive breeding on vocal behaviour have been examined in detail in a close relative of the Java sparrow, the Bengalese finch through its domestication from the white-rumped munia (*Lonchura striata*). Songs of Bengalese finches are more syntactically complex than those of white-rumped munia (Honda & Okanoya 1999) and a number of explanations for these differences have been proposed. It has been suggested that predation pressure may result in selection for shorter songs in wild environments, and, when this pressure is removed in captivity, songs can become longer and more complex in line with female preferences (Honda & Okanoya 1999). Changes in species assemblages may also influence songs. Syntactical complexity of songs in white-rumped munia was lower where mixed flocks contained sympatric related species (Kagawa et al. 2012). Naïve white-rumped munia performed songs more similar to Bengalese finches than wild white-rumped munia, suggesting that release from selection pressures related to recognition could play a role in vocal changes in captivity. Bengalese finches have lower corticosterone levels than white-rumped munia (Suzuki et al. 2012), which has been shown to influence songs in other species (zebra finch; reduced song complexity and size of song nuclei (Spencer et al. 2003)). In this way, differences in corticosterone between populations could contribute to vocal divergence. Although changes in selection pressures are likely to be more pronounced during domestication than in ex situ breeding programmes, and artificial selection may play a role, these changes give some indication of what factors could be important for vocal change during captive breeding.

8.1.4 Differences in acoustic environments

Vocalizations during ex situ breeding programmes may be influenced by properties of the acoustic environment in captivity. Captive environments differ from those in situ in a range of ways relevant for vocal communication, including sound transmission properties of enclosures and in species assemblages (Lewis et al. 2021). Changes in habitat structure, such as planting structure and the presence of smooth, man-made structures, such as windows, may alter sound transmission in the captive environment (Hughes 2007; Lewis et al. 2021; Schneider & Dierkes 2021; Clark & Dunn 2022). This may affect vocalizations through acoustic adaptation to improve signal transmission (Brown & Handford 2000; Boncoraglio & Saino 2007; Barker 2008; Brumm & Naguib 2009), or through changes in signal characteristics to avoid signal competition with other species (Ficken et al. 1974; Planque & Slabbekoorn 2007; Luther 2009; Grant & Grant 2010). Most significantly, the sound environment experienced by animals in captivity may be far removed from that experienced by their wild conspecifics (Lara & Vasconcelos 2019). Many sound sources in captivity relate to the presence of humans and anthropogenic noise sources (Clark & Dunn 2022). In Chapter 7, I determined that human presence had a significant effect on the sound environment in zoos, with louder environments and a greater proportion of anthropogenic noise during periods of normal zoo opening compared to the COVID-19 closure period. Whilst the sound environment was not specifically compared to in situ habitats, the significant effects of human presence suggests that the sound environment in zoo aviaries is likely to differ from that experienced by birds in in situ environments with less anthropogenic disturbance.

Anthropogenic disturbance can have significant impacts on vocal behaviour in birds, with birds singing louder and at higher frequencies in urban, compared to rural, environments (e.g., Luther & Baptista, 2009; Mendes et al., 2011; Nemeth & Brumm, 2009; Slabbekoorn et al., 2003). Responses to anthropogenic noise could be plastic, with vocalizations shifting dependent on the present sound environment. In white-crowned sparrows (*Zonotrichia leucophyrs*), changes in the sound environment due to COVID-19 lockdowns had a significant effect on vocal behaviour (Derryberry et al. 2020). Birds in urban territories sang more quietly and with greater frequency bandwidth in response to release from masking by low-frequency noise. However, long term anthropogenic noise disturbance may exert selection pressures on vocal behaviour resulting in long-term changes. In loud environments near airports, birds often begin singing earlier to avoid anthropogenic noise (De Framond & Brumm 2022). Following the closure of Berlin Tegel airport, many bird species shifted their onset of singing to be later, in line with conspecifics in other areas. However, a number of species still sang earlier following closure, suggesting selection for early singing individuals (De Framond & Brumm 2022). Presence of anthropogenic noise might also drive cultural change in vocal behaviour, contributing to vocal divergence. In dark-eyed juncos (*Junco hyemalis*), urban males had higher minimum frequencies than males in the mountain environment (Cardoso & Atwell 2011). Whilst there was evidence that birds modified the minimum frequency of individual memes, this only explained some of the divergence. The remaining divergence was related to the replacement of low frequency memes with higher frequency ones.

The combined evidence from other settings suggests that human influence on the acoustic environment could have significant impacts on vocal behaviour in captive environments. The acoustic environment is likely to differ for populations of the same species both within and between zoological collections. The analyses in Chapter 7 revealed differences in sound profiles and anthropogenic effects across aviaries. The acoustic environment in zoos may differentially impact certain species depending on a range of factors, such as hearing sensitivity, vocal range, and vocal inheritance. Direct examination of how the sound environment in zoos influences vocal behaviour could prove informative for management.

8.2 Effects of vocal change on conservation programmes

Throughout this thesis, I have identified a number of mechanisms that could result in vocal change during ex situ breeding programmes. In this way, populations of Java sparrows and other species may exhibit vocal divergence both from wild populations and from other captive populations. The potential influences on vocal divergence are particularly relevant for Java sparrows, as they are closed-ended learners, i.e., they do not modify their vocal repertoire after the first year (Beecher & Brenowitz 2005). As such, birds may be unable to learn population specific songs or adapt to new environments that they encounter later in life. Unfortunately, I was not able to compare multiple populations of Java sparrows to determine the extent of vocal divergence between populations, both in and ex situ. However, I uncovered clear avenues by which these differences could emerge. Given the potential for vocal divergence, the next step is to determine whether these changes matter for conservation success.

8.2.1 Mate preference

In Chapter 2, I explored how preferences for familiar songs (e.g., local dialects) could influence patterns of mating among populations of different origins. Preferences for familiar song dialects are well-reported among birds, with females showing greater responses towards familiar, rather than unfamiliar dialects (MacDougall-Shackleton & MacDougall-Shackleton 2001; Nowicki et al. 2002b; Searcy et al. 2002; Hernandez et al. 2009). For example, female song sparrows (*Melospiza melodia*) showed similar responses to their own and nearby dialects (18km), but discriminated again dialects from greater distances (>34km) (Searcy et al. 2002). Preferences for familiar songs can contribute to assortative mating, a form of mate choice in which animals select mates based on phenotypic similarity to themselves (Jiang et al. 2013). If females choose mates in a way that promotes assortative mating, this could limit reproductive success during conservation efforts. Although the extent to which population differences in vocal behaviour can result in reproductive isolation and speciation is complex and requires further investigation (Slabbekoorn & Smith 2002; Podos & Warren 2007), subtle differences in mate choice patterns could still be important in conservation programmes given the small populations involved. If females choose mates, it follows that some individuals may not be chosen, which can reduce the effective population size and affect population viability (Anthony & Blumstein 2000). Increased selectivity in small populations where mating opportunities are limited may result in fewer individuals finding suitable mates and contributes to population declines (Bessa-Gomes et al. 2003).

The effects of assortative mating during conservation interventions have been well explored in the North Island Kokako (*Callaeas wilsoni*) during multi-source translocations. Birds showed greater responses to local over foreign dialect song, suggesting a role for familiarity during interventions (Bradley et al. 2013). Over 10 multisource translocations across an 18-year period (1993-2011), birds paired assortatively with respect to origin and song dialect over most seasons and sites, with few mixed-dialect pairs forming (Bradley et al. 2014). In a more detailed examination of a single translocation, not only did few mixed-dialect pairs form, time to partnering in these pairs was considerably longer than for those with matched dialects (Rowe & Bell 2007).

In Chapter 5, I demonstrated that female Java sparrows preferred familiar over unfamiliar songs. Females showed greater responses to their father's song, or songs similar to their father's song, than to unfamiliar songs. This suggests that population differences in song phenotype resulting from vocal change during ex situ breeding programmes could influence mate choice in this species. In female Java sparrows, preferences are likely to be learned early in life. This is the case in other estrildid finches; female zebra finches removed from their father early in life did not develop preferences for father's song (Clayton 1988) and females exposed to songs of a male other than their father developed stable preferences for tutor songs (Riebel 2000). Java sparrow females' early life experiences are, therefore, likely to affect their preferences throughout life during conservation programmes. Preferences for songs from their own population, or similar to their own population, could affect pair formation and success in Java sparrows, as demonstrated in other species. Importantly, not only may females prefer birds from their own population over those of wild origin, but they may also show specific preferences between captive populations with different songs, which could reduce reproductive success during ex situ breeding programmes specifically. The effects of differences in vocal behaviour between wild and captive and among captive populations on mate choice has not been thoroughly examined in birds. However, this has been explored in other taxa. The calls of captive golden mantella frogs (*Mantella aurantiaca*) are significantly different from those of wild frogs, and zoo-bred frogs showed a stronger response to playback of calls from their own population than to calls of wild origin (Passos et al. 2017).

Whilst I have demonstrated that familiarity is an important component of song preference in Java sparrows, overall mate preference may also be influenced by other features. Among other species, females show preference for a range of song traits including, complexity (e.g., Leitão et al., 2006), temporal features (e.g., Dunning et al., 2020; Nolan & Hill, 2004), demonstration of learning ability (e.g., Nowicki et al., 2002b), and consistency (e.g., Woolley & Doupe, 2008). In Java sparrows, there is also evidence that females show a preference for larger males (Hasegawa et al. 2011). For this reason, real world mate choice may be complex and affected by more factors than familiarity alone. This does not mean that familiarity is not an important feature in more natural settings. In zebra finches, individuals within a single population mated assortatively based on the song culture of their foster grandparents, and pairings were random with respect to genetic variation in body size (Wang et al. 2022). As well as vocal behaviour affecting mate choice, females may also exert post-mating sexual selection. In Bengalese finches, females produced eggs with greater mass and tended towards male-biased sex rations when paired with males with longer song durations, which could indicate quality (Soma & Okanoya 2013). The evidence provided in Chapter 5, demonstrating preference for familiar songs, is a useful first step for informing Java sparrow conservation. However, further exploration of factors affecting mate choice and reproductive success are necessary to fully understand the consequences of vocal behaviour in conservation programmes.

8.2.2 Responses to other vocal behaviour

As well as altered responses to mating signals, animals in captive environments may have altered responses to other, survival relevant, vocal signals such as alarm calls and those for territory defence and maintenance (e.g., Friant et al., 2008; Sabol et al., 2022). Hawaiian crows in conservation breeding programmes were still able to distinguish and respond to alarm and territorial inclusion calls (Sabol et al. 2022), despite evidence that these calls were less frequently used in captive individuals (Tanimoto et al. 2017). However, individuals differed in their levels of response to calls, with some birds not responding to playbacks, suggesting these birds may have become desensitized to important calls due to changes in the social and physical environment (Sabol et al. 2022). Altered responses to survival relevant calls have also been reported in other taxa. Captive-born cotton-top tamarins (*Saguinus oedipus*) responded similarity to vocalizations from predator and non-predator species, perhaps responding to vocal qualities instead (Friant et al. 2008).

The detailed examination of aspects of vocal behaviour outside of song and mate choice was beyond the scope of this thesis. However, the maintenance of appropriate species-specific behaviour and responses is an important consideration during ex situ breeding programmes. Disruption of signaller-receiver interactions may result in inefficiencies in communication that challenge conservation goals (Sabol et al. 2022). Because of this, further investigation into the effects of ex situ breeding programmes on parts of the vocal repertoire other than song is also important in designing effective programmes.

8.3 Implications for captive management of birds and other species

Potential mitigations for the influence of vocal change in ex situ breeding programmes were not directly explored in this thesis. However, based on the mechanisms and effects highlighted above, and results from other studies, it is possible to identify potentially useful strategies that can be incorporated into management.

Throughout this thesis, I have demonstrated that captive management and ex situ breeding programmes have the potential to influence vocal behaviour and promote vocal divergence between populations, and this can influence important behaviours, such as mate choice. Therefore, the first important step is to consider vocal behaviour and the potential for vocal change in conservation breeding programmes. An essential part of this is understanding the life history and vocal behaviour of the species in question. In Chapter 2, I identified a range of features that could increase the possibility of vocal divergence and the level of impact this may have. This included the mechanisms affecting song inheritance, with vocal learners being particularly susceptible to divergence, when song is learned, with closed-ended learners being less able to alter vocalizations and adapt to new environments later in life, and aspects of species' vocalizations, such as complexity, which can alter rates of vocal divergence. This information could be used to identify species especially at risk of issues relating to vocal behaviour. Ideally, conservation practitioners would be able to locate this information in current literature. However, for rare or understudied species, further research into vocal behaviour may be required.

Reducing the length of (generational) time birds spend in captivity could help to reduce the effects of vocal divergence. Whilst some level of vocal change can happen in a single generation due to cultural processes, e.g., the introduction of copying errors or improvisation during learning, significant population differences may take longer to establish. In addition, changes in vocal behaviour related to selection, such as acoustic adaptation or inadvertent selection for other traits, are likely to become more pronounced over time. In golden mantella frogs, differences were apparent between the wild population and two ex situ populations (Passos et al. 2017). However, calls from the ex situ population with lower generational time were more similar to wild calls. Guidelines for establishing ex situ management programmes suggest that projects should have a clear and appropriate time frame (IUCN Species Survival Commission 2014), and potential for vocal divergence could be incorporated into these decisions. However, many species have already been housed in captive environments for significant lengths of time and may already be significantly diverged from wild populations (Passos et al. 2017; Tanimoto et al. 2017).

Increasing environmental similarity between wild and captive environment has been suggested as a means of reducing inadvertent selection during ex situ breeding programmes (Shuster et al.

2005; Frankham 2008; Williams & Hoffman 2009). Similarly, increasing similarity in acoustic environments could reduce vocal divergence related to acoustic adaptation and signal masking. Chapter 7 indicated a significant effect of human presence on the sound environment in aviaries, and reducing this contribution may positively influence the soundscape. Sound mitigation has been explored from an animal welfare perspective in some species (e.g., Orban et al., 2017). Sound-absorbing barriers proved successful in reducing noise produced by an HVAC (heating, ventilation, and air conditioning) system for a female anteater (Orban et al. 2017). It may, therefore, be possible to reduce the impacts of anthropogenic noise on the acoustic environment in zoos through relatively simple interventions. Different materials of sound absorber had different levels of effectiveness, and this differed between frequency bands (Orban et al. 2017). In this way, it may be necessary to consider species' biology and hearing when choosing materials. Playback of ecologically relevant sounds has also been investigated. Playback of rainforest sounds appeared to increase agitation in western lowland gorillas (*Gorilla gorilla gorilla*) (Ogden et al. 1994). Adding sound to enclosures should be considered carefully, as it has the potential to alter behavioural patterns in a negative way. It is not clear if playback would influence the vocal behaviour of individuals. However, playback of ecologically relevant sounds may be useful for other aspects of zoo management, such as encouraging breeding behaviour through the playback of conspecific calls (O'Connell-Rodwell et al. 2004; Clark et al. 2012).

Reduced learning opportunities were highlighted as a potential cause of vocal change during ex situ management. Therefore, increasing potential learning opportunities could reduce the likelihood of vocal change or limit its impacts. Exposing birds to conspecific songs could increase learning opportunities and improve the success of conservation interventions, such as reintroductions. In the regent honeyeater, juvenile birds that had experienced song tutoring from adult, breeding birds (via a live feed) had increased survival post release (Tripovich et al. 2021). However, the effects on breeding success were less clear; males exposed to conspecific song had a lower chance of breeding success, although this was no longer apparent when only males resighted during the breeding season were included. Puerto Rican parrots (*Amazona vittata*) moved between captive populations acquired local signals in their new populations (Martínez & Logue 2020). As such, it was suggested that familiarization of birds with new dialects during conservation programmes may facilitate transmission of vocal signals and improve conservation outcomes in this species. Familiarising young birds with songs from other populations could also be used to influence mate selection. Female white-crowned sparrows from mixed-dialect populations were not more likely to choose mates with the same dialects as their fathers and did not show consistent preferences across successive breeding seasons (Chilton et al. 1990). When tested in captivity, birds did not show differences in response to either of the dialects they commonly heard (Chilton et al. 1996). It is possible that playback of songs could be used to increase familiarization with other dialects prior to mixing or release, reducing the extent of assortative mating based on population of origin.

Finally, it may be possible to screen individuals or populations during conservation programmes to increase the likelihood of success. This method was examined during the release of cactus parakeets (*Eupsittula cactorum*) recovered from anti-trafficking operations (Martins et al. 2018). It is not often possible to determine the original locality of individuals recovered from trade, so other methods to define suitable release sites would be beneficial. Geographic dialects were examined to infer origins and determine which release sites would have been most appropriate for reintroductions based on the recovered individuals' calls. Such methods would help to reduce acoustic contrasts between released individuals and wild populations, potentially increasing the success of reintroductions due to the importance of effective communication (Wright et al. 2008). As well as screening populations, screening individuals involved in conservation programmes may also be a useful method. Given the variation in individual responses to survival relevant calls in Hawaiian crows, it was suggested that not all individuals would be equally well-equipped for release to the wild (Sabol et al. 2022). Those that did not exhibit adequate responses to survival relevant calls may have reduced survival post-release. In a similar way, responses to mating signals of wild or other captive populations could be used when selecting individuals to be moved between populations, with greater responses signalling greater probabilities of success. Screening individuals is likely to be resource and time intensive. As such, this approach may be better suited to small-scale interventions, such as those involving single or few individuals, rather than large scale programmes.

8.4 Future directions

This thesis provides an initial examination into the role of vocal behaviour in ex situ breeding programmes, focusing specifically on a model species, the Java sparrow. It also highlights some key areas for further research.

Firstly, the extent to which vocal divergence between wild and captive populations, and among captive populations, has already occurred should be determined. This has been achieved for some taxa (e.g., golden mantella (Passos et al. 2017), Hawaiian crow (Tanimoto et al. 2017), Puerto Rican parrot (Martínez & Logue 2020)). However, differences in life history, vocal parameters, and vocal inheritance are all likely to influence the magnitude and rate of observed differences. Therefore, expanding these investigations to a wider spectrum of species and taxa would be beneficial in developing broad strategies for examining and combatting vocal change during ex situ breeding programmes.

Secondly, after improving our understanding of vocal change during ex situ management, incorporating vocal behaviours into management plans, including potential mitigations against negative effects, may help to increase success in both in and ex situ conservation programmes. To achieve this, further investigation into mitigation measures, such as reducing impacts of anthropogenic noise on the acoustic environment and the use of playbacks and screening, is required.

Finally, there has been a recent increase in interest in the sound environment in zoological collections (Pelletier et al. 2020; Rose et al. 2021; Clark & Dunn 2022). Given the potential role of the zoo soundscape for animal behaviour, welfare, and vocal change, a better understanding of the acoustic environment in zoos would be beneficial to captive animal management. However, there is currently little consistency in the methodology for examining environmental sound in captivity (Clark & Dunn 2022). Further investigation into the sound environment in zoos using a range of metrics, including sound pressure levels and acoustic indices (Sueur et al. 2014; Clark & Dunn 2022) could help to provide a more holistic assessment of the soundscape in zoos and its importance for management.

8.5 Conclusions

This thesis set out to answer two questions; do mating traits evolve ex situ, and should we care? The combined evidence from this body of work suggests that the answer to both of these questions is yes. Firstly, I identified a range of factors that could impact vocal divergence during ex situ breeding programmes, including cultural processes, changes in other, correlated traits, and differences in the acoustic environment. I found that these factors were likely to affect vocal divergence in a model species, the Java sparrow. Following this, I went on to examine the second question, finding that vocal divergence has the potential to influence the success of conservation programmes both in and ex situ by affecting mate choice, promoting assortative mating. It is, therefore, important to consider vocal behaviour during conservation programmes and to investigate methods to reduce its potential negative impacts on conservation success.

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Appendix 1: Information on subjects and housing

A number of chapters in this thesis use archival recording data from a laboratory population of Java sparrows (Lonchura oryzivora) housed at Hokkaido University. Archival data used in this study was collected between 2011 and 2019. The age of birds at recording ranged from 0.41 to 8.83 years.

Across the population birds were housed at a temperature of 25 ± 3 °C, humidity 30 – 60 %, with a 12L:12D lighting schedule. Birds are provided with food (mixture of seeds (3:1:1:1 mix of Japanese millet, fox tail millet, millet, and canary seed), shell grit, and fresh green vegetable) and water ad libitum. Unless breeding, nesting materials were not habitually provided to prevent birds from becoming unnecessarily motivated for breeding and to reduce the probability of egg binding. Cages were serviced weekly.

Birds in this population are usually housed in large single sex cages (43 × 37 × 41 or 46.5×46.5×94cm) with up to 20 individuals. Cages are kept in a single room, and are neither visually nor audibly isolated from one another. During breeding, family groups (a single pair and their offspring) are housed in individual cages (43 × 37 × 41cm) which are visually, but not audibly, isolated from other cages. A small number of chicks were also housed with an additional male bird that was not their social father, which acted as a second song tutor. Juveniles remained in their family cage until ~180 days of age.

During recording of males, birds were housed singly in a small cage (27 × 36 × 18cm), which was placed in a soundproof recording chamber to ensure recordings contained only songs from a single individual with a high signal-to-noise ratio, as this is important for analysis. To ensure sufficient recordings are obtained for analyses, individuals may remain in the recording chamber for up to 72 hours, although if enough recordings are obtained in a shorter time period, birds are removed from the recording chamber and returned to their usual environment. Most birds spend <24 hours in the recording chamber. Food and water were provided ad libitum during this time. Individuals are not strongly bonded with cage-mates. As such, single housing for this time period is unlikely to have detrimental effects on birds' welfare. Birds were checked regularly during single housing to ensure that they did not show signs of distress. Following the recording period, birds were returned to their usual habitat.

During playback tests with females, birds were housed singly in a small cage (27 × 36 × 18cm) for the duration of the experimental period. Cages were kept together, so individuals were not audibly or visually isolated from other birds except when completing an experimental trial or habituation period. Individuals were placed in a soundproof chamber the day prior to completing a playback trial, and were placed back in social contact with other birds following completion of the trial. As such, birds were isolated for <12 hours at a time. As previously, birds are not strongly bonded to their cage mates, and were not socially isolated from other individuals for long periods, so single housing for this time period is unlikely to have detrimental effects on birds' welfare. Birds were checked regularly during single housing to ensure that they did not show signs of distress. Following the experimental period, birds were returned to their usual habitat.