



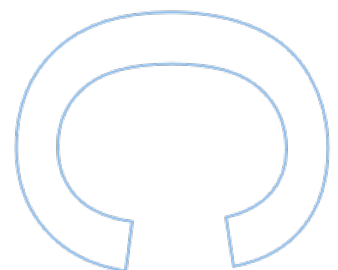
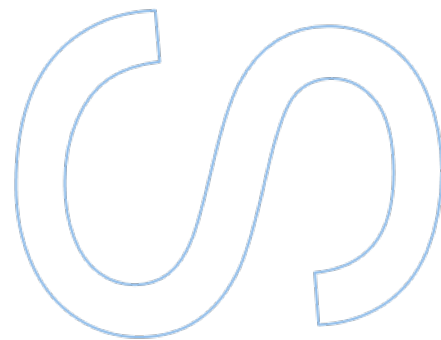
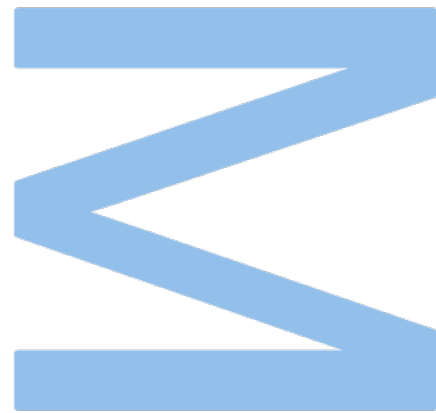
Behavioural laterality and social structure in common waxbills

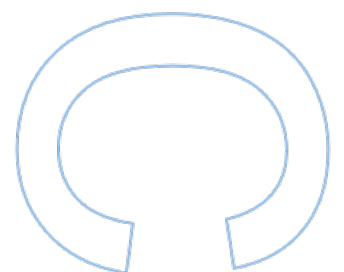
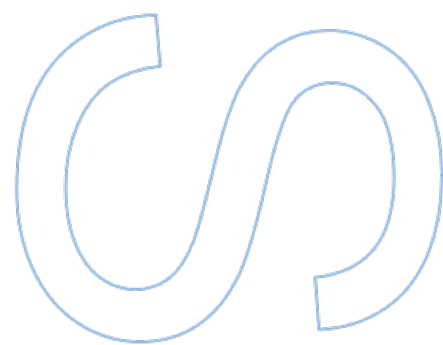
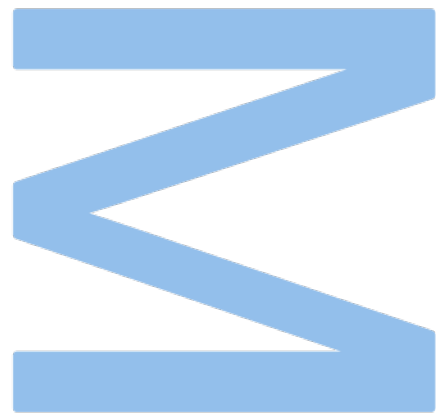
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Pedro Miguel Miranda Santos

Porto, 30 de setembro de 2022

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Resumo

A lateralização comportamental é uma característica que se encontra em vários taxa, de invertebrados a vertebrados. Esta consiste em usar preferencialmente um lado do corpo, como um dos membros ou um dos olhos, por exemplo, ao realizar determinados comportamentos. A lateralização comportamental pode estar relacionada com especialização dos dois hemisférios cerebrais para desempenhar funções específicas e, provavelmente por causa disso, indivíduos mais lateralizados podem ter melhores capacidades cognitivas e melhor desempenho em vários contextos ecológicos (por exemplo, localização de alimentos, interações predador-presa, e acasalamento). Apesar das aparentes vantagens da lateralização comportamental, diferenças na força e direção de lateralização podem ser encontradas entre indivíduos da mesma espécie e população. De facto, estudos anteriores sugeriram que esta variação poderia estar relacionada com diferenças individuais noutros traços, como tipo de personalidade, stress, sexo, ou com diferenças no contexto ecológico. Para entender melhor as consequências das diferenças individuais na lateralização comportamental, eu estudei uma população selvagem e extensivamente fenotipada de bicos-de-lacre (*Estrilda astrild*), usando um setup experimental que me permitiu quantificar se cada indivíduo se posicionava preferencialmente com o lado esquerdo ou direito para se alimentar. Os meus objetivos foram 1) quantificar a direção e a força de lateralização comportamental nos diferentes indivíduos, 2) testar lateralização ao nível da população, 3) investigar se as diferenças de lateralização estavam associadas a diferenças morfológicas e comportamentais e 4) testar se bicos-de-lacre com medidas de lateralidade diferentes ocupavam posições diferentes na sua rede social. Com este estudo foi-me possível encontrar evidência de lateralização ao nível populacional, uma vez que a maioria dos bicos-de-lacre escolheu posicionar-se com o lado direito para se alimentar, e mostrar também diferenças na direção e força de lateralização entre os indivíduos. A direção da lateralização comportamental foi associada ao desempenho dos indivíduos num “detour-reaching task”, que avalia a capacidade de controlo inibitório. Essa associação foi mais clara no sexo masculino, onde os indivíduos mais lateralizados à esquerda foram os que apresentaram melhor controlo inibitório. As fêmeas mostraram ser, em média, mais lateralizadas à direita do que os machos. Não encontrei associações entre a força ou a direção da lateralização e outros fenótipos estudados, incluindo tamanho corporal, tipo de personalidade, sinais de stress, dominância social ou

centralidade na rede social. Estes resultados indicam conexões entre a lateralização comportamental e as capacidades cognitivas, em particular com a capacidade de controlo inibitório. Enquanto a maioria dos estudos anteriores reportaram uma relação entre cognição e a força de lateralização comportamental, os meus resultados estão entre os poucos que indicam que a direção da lateralização também pode estar associada com o desempenho cognitivo. Finalmente, estes são os primeiros resultados que indicam uma diferença entre sexos na relação com a lateralização comportamental e o desempenho cognitivo, o que mostra a importância de considerar o sexo ao estudar a lateralização comportamental.

Palavras-chave: lateralização comportamental, sexo, controlo inibitório, personalidade, morfologia, comportamento social, bico-de-lacre

Abstract

Behavioural lateralization is widespread across animal taxa, from invertebrates to vertebrates. It consists of preferentially using one side of the body, a left- or right-side limb or eye for example, when performing certain behaviours. Behavioural lateralization can be related to specialization of the two brain hemispheres to perform specific functions and, likely because of this, more lateralized individuals have been found to have better cognitive capabilities and perform better in various ecological contexts (e.g., foraging, predator-prey interactions, and mating). Despite the apparent advantages of lateralization, differences in strength and direction of behavioural lateralization can be found between individuals of the same species and populations, which previous studies have suggested to be related with individual differences in other traits, such as personality-type, stress, sex, or with differences in ecological context. To better understand the consequences of individual differences in behavioural lateralization, I studied a wild-caught and extensively phenotyped population of common waxbills (*Estrilda astrild*), using an experimental setup to quantify whether each individual preferentially used its left or right side to feed. My goals were 1) to quantify the direction and strength of behavioural lateralization in the different individuals, 2) to test for population-level lateralization, 3) to investigate if differences in lateralization were associated with differences in morphological and behavioural phenotypic traits, and 4) to test whether waxbills differing in laterality occupied different positions in their social network. I found evidence of population-level lateralization, since most waxbills choose the right side to feed, but differences in the direction and strength of lateralization were present between individuals. The direction of behavioural lateralization was associated with performance in a detour-reaching task, which assays inhibitory control ability. This association was clearer in males, where more left-side lateralized individuals were the ones with better inhibitory control. Females were on average more right-side lateralized than males. I did not find associations between the strength, or the direction of lateralization and other phenotypes studied, including body size, personality type, cues of stress, social dominance, or centrality in the social network. These results indicate connections between behavioural lateralization and cognitive capabilities, in particular with inhibitory control ability. While most previous studies found relationships between cognition and the strength of behavioural lateralization, my results are among the seemingly few (e.g., Bibost & Brown, 2014; Lucon-Xiccato et al., 2020) indicating that the direction of lateralization may also be

associated with cognitive performance. Finally, to my knowledge these are the first results indicating a sex-specific association between behavioural lateralization and cognitive performance, which highlights the importance of considering sex when studying behavioural lateralization.

Keywords: behavioural lateralization, sex, inhibitory control, personality, morphology, social behaviour, common waxbill

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List of abbreviations

LI – Lateralization index

ALI – Absolute lateralization index

GLM – Generalized linear model

AICc – Akaike's information criterion (corrected for small sample sizes)

PIT – Passive integrated transponder

RFID – Radio frequency identification

CV – Coefficient of variation

MCMC – Markov chain Monte Carlo

1. Introduction

Once thought to be a distinctive human feature, behavioural lateralization is nowadays known to be a widespread trait across animal taxa (Berg et al., 2020; Frasnelli & Vallortigara, 2018; Rogers & Kaplan, 2019). Behavioural lateralization has been documented, for example, in mammals (McGrew & Marchant, 1997; Piddington & Rogers, 2013; Siniscalchi et al., 2017), birds (reviewed in Rogers & Kaplan, 2019), fish (C. Brown et al., 2004; Lucon-Xiccato et al., 2020; Reddon & Hurd, 2009), and several invertebrate species (reviewed in Frasnelli, 2013). It can be manifested by various types of behavioural asymmetries in animals, such as preferences in the use of right or left sensory organs, or limbs, when performing specific tasks (Brown & Magat, 2011; Magat & Brown, 2009). Such behavioural asymmetries can be associated with functional specialization of the nervous system by means of “cerebral lateralization” (Chivers et al., 2017). Cerebral lateralization is considered advantageous since it allows different types of cerebral functions to be separated, and different stimuli to be processed separately by the two hemispheres (Whiteside et al., 2018), increasing neural efficiency and enabling “multitasking” by operating on different types of information at the same time (Brown & Bibost, 2014; Frasnelli & Vallortigara, 2018; Ghirlanda & Vallortigara, 2004; Güntürkün et al., 2000; Magat & Brown, 2009). Division of functions between the two sides of the nervous system can be easily observed in animals with large monocular visual fields, such as birds, who need to separately use their right and left eyes in order to perceive visual stimuli (Frasnelli & Vallortigara, 2018; Rogers & Kaplan, 2019). Since the lateral part of the retina of each eye is connected to the contralateral hemisphere, input from the right eye is mainly processed by the left hemisphere, and the left eye by the right hemisphere (Frasnelli & Vallortigara, 2018; Rogers & Vallortigara, 2015). In one of the most extensively studied species regarding preferential hemisphere use during specific tasks, chicks of the domestic chicken (*Gallus gallus*) perform better at discriminating pebbles from grain when using their right eye/left hemisphere, and when right-eye usage is experimentally disturbed and only the left eye is available, chicks lose discrimination abilities and increase their copulation and attack levels (Mench & Andrew, 1986; Rogers et al., 1985). Furthermore, chicks are better at perceiving predator cues that appear from their left-side, thus exhibiting a left eye/right hemisphere dominance during vigilance behaviour (Dharmaretnam & Rogers, 2005). Studies of visual lateralization conducted on other

species, such as the Pigeon (*Columba livia*; Güntürkün et al., 2000) and Zebra finch (*Taeniopygia guttata*; Alonso, 1998), for example, contributed to find a general pattern where the right eye is specialized in object categorization and discrimination, as well as the one most used under relaxed conditions, whilst the left eye is dominant under stressful and emotionally charged situations, such as copulation, predator detection and escape activity (Rogers, 2021). Behavioural lateralization can vary in its direction (individuals can be biased in their preference towards the right or left side) and strength (individuals can be more or less strongly lateralized towards one side; (Frasnelli & Vallortigara, 2018), with several studies reporting differences in these aspects of lateralization between species, between individuals of the same population, and even within-individuals depending on the task performed (Brown & Magat, 2011; Frasnelli & Vallortigara, 2018; Ghirlanda & Vallortigara, 2004; Schiffner & Srinivasan, 2013). For example, a study testing male Budgerigars (*Melopsittacus undulatus*) in five different tasks, including foot choice to climb into a perch or deciding on which side to land, showed that individuals differ in both the direction and strength of side-lateralization, and even the same individual could have different high standard deviation of lateralization strength among the different tasks (Schiffner & Srinivasan, 2013). In the European green lizard (*Lacerta viridis*), right-biased, left-biased, and non-biased individuals were found when evaluating preferential escape directions, by simulating a predator attacking individuals from behind (Pikalík et al., 2021).

Cerebral lateralization has often been linked to enhanced cognitive abilities (Frasnelli & Vallortigara, 2018; Magat & Brown, 2009; Roche et al., 2020; Rogers & Kaplan, 2019), and differences between more or less lateralized individuals can have fitness consequences (Brown & Magat, 2011; Dadda et al., 2010; Magat & Brown, 2009). For example, chimpanzees (*Pan troglodytes*) exhibit different hand preferences for fishing termites, and those more strongly lateralized (i.e., with a stronger preference for either hand) perform better at this activity (Brown & Magat, 2011; McGrew & Marchant, 1997). Similarly, common marmosets (*Callithrix jacchus*) with stronger hand preferences while foraging are faster at identifying a “model predator” while feeding, showing quicker vigilance responses than less lateralized individuals (Piddington & Rogers, 2013). It has also been shown that strongly lateralized cats (*Felis catus*) for the use of a preferred paw during a problem-solving task, namely opening a lid to access food, were more successful than ambidextrous individuals (Isparta et al., 2020).

Evidence from various taxa suggests that having a strongly lateralized brain also enhances the capacity of individuals to successfully approach tasks in a variety of ecological contexts, such as foraging, predator-prey interactions and social dynamics

(Berg et al., 2020; Rogers & Kaplan, 2019). In a study conducted with juvenile ambon damselfish (*Pomacentrus amboinensis*), individuals differed in the degree of response to predator cues depending on whether they were lateralized or not. Once predator odour was detected, lateralized individuals (either with a left or right turning bias) exhibited a much stronger behavioural response, reducing feeding and activity levels, while non-biased individuals preserved feeding and activity patterns (Chivers et al., 2017). These results suggest that strongly lateralized damselfish are cognitively more capable of quickly detecting and reacting to potential predators, possibly increasing its survival (Chivers et al., 2017). Another example is that of the shiner perch (*Cymatogaster aggregata*), where the degree of lateralization affected their escape performance, with strongly lateralized individuals showing smaller latency responses to a mechanical stimulus when compared with non-lateralized fish, suggesting a higher ability of escaping from real predators (Dadda et al., 2010). In the Gouldian finch (*Erythrura gouldiae*), a highly polymorphic species where individuals typically choose mates with the same head colour, mate choice preference is strongly lateralized in the right eye/left hemisphere complex (Templeton et al., 2012). Specifically, black-headed males preferentially responded to black-headed females only when the right eye, or both eyes were seeing, but when only the left-eye was seeing, males lost the ability to distinguish between black-headed males and females and started choosing females of different head colour (red-head polymorphs; Templeton et al., 2012). For this particular species, choosing the wrong mate can lead to reproduction failure due to genetic incompatibility, thus preferential eye-use can be considered advantageous (Templeton et al., 2012).

In light of previous arguments supporting the advantages of being strongly lateralized, it can be quite puzzling to understand why non-lateralized or weakly lateralized individuals still persist in nature. If one considers the equal probability that any given stimulus has of appearing from either side of the animal body, perhaps possessing just one side of the nervous system specialized in a certain activity would not be advantageous (Vallortigara & Rogers, 2005), creating a negative selection pressure for behavioural lateralization (Dadda et al., 2009; Lucon-Xiccato et al., 2017; Vallortigara & Rogers, 2005). For example, despite the presence of strong lateralization in the use of limbs, pheasants (*Phasianus colchicus*) with stronger biases in footedness were the ones with lowest survival rates when released into the wild (Whiteside et al., 2018). Poeciliid fish (*Brachyraphis episcopi*) from high predation environments, who showed significant biases and were better in identifying predators with the right-eye, also had more difficulty navigating through a maze (Brown & Braithwaite, 2005). Even though

strongly lateralized juvenile ambon damselfish (*Pomacentrus amboinensis*) were faster to identify potential predators nearby when compared to non-lateralized fish, as noted before, they were also worse competing with conspecifics, since strongly lateralized individuals were more likely to be attacked by others than non-biased individuals (Chivers et al., 2017). Perhaps behavioural laterality can be beneficial in certain situations but disadvantageous in others (Dadda et al., 2009). Under this scenario, one can assume that a possible equilibrium-point between being strongly lateralized or non-lateralized, may depend on the type of behaviour, species, or sex studied, and even on the individuals' stress levels (Rogers, 2021). Importantly, the general pattern found both within and across species is that behavioural lateralization is more often present than absent, and it can be found from insects to humans, suggesting that behavioural lateralization advantages may outweigh its costs (Vallortigara & Rogers, 2005).

Most research has generally shown advantages of being lateralized, irrespective of the direction of lateralization (i.e., left or right; Frasnelli & Vallortigara, 2018; Rogers, 2021). For example, the case of the chimpanzees mentioned earlier illustrates this point, since the individuals that were better at fishing termites were the ones with more strongly lateralized handedness, independently of its direction (McGrew & Marchant, 1997). Hence, it could be expected that an equal proportion of left and right-biased individuals exist in wild populations, but, contrary to this expectation, the most common situation is that the majority of individuals in a population are biased towards the same direction (Ghirlanda & Vallortigara, 2004). This is referred to as population-level lateralization (Denenberg, 1981), and in general the majority of individuals biased in the same direction is around 60-90% (Frasnelli & Vallortigara, 2018; Ghirlanda & Vallortigara, 2004). Why such an unbalance in the direction of laterality exists in populations can be complicated to understand. It can even be argued that, if the majority of individuals in a population is biased towards the same direction, predators would be able to predict where the group would flee, presenting a potential disadvantage (Vallortigara & Rogers, 2005).

In an attempt to shed some light into the matter of population-level lateralization, Ghirlanda and Vallortigara (2004) demonstrated, through the construction of a game-theoretical model, that populations consisting of an uneven number of right and left biased individuals can be stable and evolve, if being lateralized in either direction has frequency-dependent costs and benefits. For example, models showed that during predator-prey interactions, it would be advantageous for the majority of individuals in social species to align their asymmetrical biases, in order to increase cooperation, decrease predation, and maintain cohesion within groups (Frasnelli & Vallortigara,

2018). This mathematical model was later adapted to consider intraspecific interactions between individuals, such as cooperation and competition (Ghirlanda et al., 2009), showing that when selective pressures towards group synchrony increased, individuals would coordinate their activities and, therefore, the number of individuals with the same bias would also increase (Ghirlanda et al., 2009). As for empirical evidence supporting this hypothesis, it has been demonstrated in several species of social bees (*Apis mellifera*; *Bombus terrestris*; *Trigona carbonaria*; *Trigona hockingsi*; *Austroplebeia australis*) that population-level lateralization is present for recalling olfactory memories with either their right or left antennae, contrarily to solitary bees (*Osmia rufa*; Frasnelli & Vallortigara, 2018; Frasnelli et al., 2011). Also, Giant Australian cuttlefish (*Sepia apama*) show a preference to view other male contestants with their left-eye when fighting, but individuals with higher success rates are the minority who use their right-eye, exactly because these minority right-eye individuals can surprise their contestants by exhibiting a behaviour the majority are not accustomed to (Schnell et al., 2019). Additionally, in a context of mating where individuals must coordinate their behaviours, males from this species tended to view females with the left-eye and approach them from the right-side, and in this case, individuals that chose this side possessed higher mating success because they were more probable of being accepted by females (Schnell et al., 2019).

The social nature under which population-level laterality is thought to have originated from, together with previous work relating laterality and social interactions (Anfora et al., 2010; Daisley et al., 2009; Giljov & Karenina, 2019), suggests a possible strong link between social behaviour and laterality. Up to this day, few studies investigated if behavioural lateralization could shape the structure of social networks or influence an individual's centrality in a social network or their hierarchy ranks. One of those studies investigated how manipulating the development of cerebral lateralization in chicks of the domestic chicken, affected the organization of social structure: chicks from eggs reared in darkness did not develop brain asymmetries and formed a less stable group structure, with individual hierarchy rankings varying between days (Rogers & Workman, 1989). More recently, the relationship between behavioural lateralization and social network structure has been investigated in chimpanzees, by testing if individuals associated more with others of similar hand-biasing while grooming, or if right-handed chimps occupied more central positions in the network, but no significant relationship was encountered in either case (Díaz González, 2021). Despite these valuable contributions, studies regarding this subject are scarce and further investigation is required.

Additionally, several studies looked into a possible bridge between behavioural lateralization and animal personality (i.e., a set of behavioural traits that differ consistently between individuals across time and different contexts; Goursot et al., 2019). For instance, in a study conducted with a freshwater fish, the convict cichlid (*Archocentrus nigrofasciatus*), individuals with stronger behavioural lateralization exploring a familiar environment were also the ones exhibiting bolder personality when exploring a novel one (Reddon & Hurd, 2009). Right-biased pigs (*Sus scrofa*), have also been shown as bolder, more explorative and more sociable when compared with left-biased individuals (Goursot et al., 2019). Finally, strongly lateralized Port Jackson sharks (*Heterodontus portusjacksoni*) were more stressful than weakly lateralized individuals (Byrnes et al., 2016).

Stress and behavioural lateralization seem to share a contradictory and unresolved relationship, with several studies reporting how on one hand stress can change the functional lateralization of the brain over time, and on the other hand, how being lateralized can affect an individual's stress reactivity (Ocklenburg et al., 2016). For example, Morgante et al. (2007) showed that strongly lateralized female sheep (*Ovis aries*) develop a higher hormonal stress response induced by lamb separation, compared to weakly lateralized female sheep. Another study conducted with chicks of the domestic chicken demonstrated that the administration of corticosterone (stress hormone) during embryonic stages reduced the development of visual lateralization processes, causing chicks to take longer to identify a predator image while searching for grain in a mix of pebbles, in contrast with faster (normally asymmetric) individuals who were treated 1 day after hatching (Freire et al., 2006).

To this day the observed variability in the degree of behavioural lateralization (both in captive and wild populations), as well as the possible factors shaping such variance, remain somewhat unresolved (Berg et al., 2020; Byrnes et al., 2016). Here, I studied the behavioural lateralization of the Common waxbill (*Estrilda astrild*), a small, highly gregarious finch species that favours open habitats such as savannas and areas containing tall grasses where it forages and roosts (Cardoso & Reino, 2018). The waxbills here studied have previously demonstrated individual differences in cognition, personality, stress, and dominance rank (amongst others; see Beltrão et al., 2021; Gomes et al., 2020; Guerra et al., 2020), phenotypes for which a relationship with behavioural lateralization has been encountered in several other species. Based on previous literature, I expect to find such a relationship in the present study, namely between the strength of lateralization and these phenotypes, which could lead to possible ecological implications for this species and advance the general knowledge on

what might cause the found variation in lateralization. Specifically, I expected that more strongly lateralized individuals had better cognitive capabilities than weakly lateralized ones. Regarding associations with personality traits, stress indicators, sex and social dominance, it was not possible to make clear predictions since results from studies on other species appear contradictory. Furthermore, waxbills are a highly social species (Harris & Davis, 1993; Payne & del Hoyo, 2010) and since it was shown that this particular population has a long-term stable social network structure (Gomes et al., 2022), I will study, perhaps for the first time in a wild avian species living in a more naturalistic environment, the relationship between behavioural lateralization and social network structure. My specific goals for this thesis were to (1) quantify the direction and strength of behavioural lateralization in the different individuals, (2) test for population-level lateralization, (3) investigate if differences in lateralization are associated with differences in morphological and behavioural phenotypic traits, and (4) to test whether waxbills differing in laterality occupy different positions in their social network. These tests will provide new insight on the nature of individual variations in laterality, advancing our understanding of the possible causes and consequences of differences in behavioural laterality.

2. Materials and Methods

2.1. Study System

I worked with a population of 68 waxbills living in semi-natural conditions in a large outdoor mesocosm at CIBIO, in Vairão, Portugal. Briefly, the mesocosm consisted of a large outdoor aviary (ca. 235 m², 1.30 – 2.70 m high) covered in fine net, with abundant natural vegetation, and connected via a small window to an indoor dormitory room (4 m², 2.25 m high). Birds were exposed to natural climate and could move freely within this space and perform their natural fission-fusion group dynamics. The majority of individuals in the mesocosm were captured during October 2016 and September 2017, in agricultural fields within a radius of 20 km from CIBIO (northwest of Portugal), which is within the invasion range of the species in the Iberian Peninsula (see Gomes et al., 2020 and Guerra et al., 2020 for capture details). From 2019 on, juveniles born in the mesocosm and some individuals added from the wild (17 waxbills) helped maintain a stable flock size of ca. 60. All individuals in the aviary have a passive transponder identifier (PIT) tag in a plastic leg ring, which enables continuous monitoring as the birds move near an array of radio-frequency identification (RFID) antennae. A total of 12 feeders, with one RFID antenna each, and 8 long perches (15.5 cm long), each with 4 small RFID antennae below, were in a feeding area inside the mesocosm. When a bird arrives at any feeder or perch, the RFID system registers its identity, its time of arrival and departure. An indoor dormitory room, to which birds have access through the mesocosm, was also equipped with a pair of RFID antennae around the entrance, so that entries and exits from the dormitory could be monitored. Maintenance of the mesocosm was kept to a minimum disturbance: birds were provided *ad libitum* seeds (Versele-Laga Prestige: Tropical finch) in feeders, water in 2 large dispensers, and 3 to 4 long and shallow containers for bathing, replenished twice a week.

2.2. Data collection and quantification of behaviour

For this experiment, data collection consisted in repeatedly filming waxbills feeding from an adapted feeder, designed so that birds had to approach the food either with their right or left side, but not frontally. Unlike the other feeders, this adapted feeder did not have a support parallel to the feeder for the birds to access food in their front. Instead, the adapted feeder had one of the regular perches, with 4 RFID antennae underneath, placed perpendicularly to it (**Figure 1**), so that waxbills had to land on the perch and then approach the food with either the left or right side of the body turned to the feeder. Birds were already habituated to this perch before data collection started, as it is one of the 8 identical perches already present in the mesocosm as part of their RFID system. The RFID antennae underneath this perch allowed constant monitoring and identification of bird's visits to the feeder.

To observe which side the birds chose to approach the feeder, I made video recordings using one small camera (Xiaomi Yi 2K Action Camera) placed under the net ceiling of the aviary to film this adapted feeder and perch. Sessions of video-recordings were distributed in time since May 12th 2021 and ended on May 31st 2022, which allowed me to quantify behavioural lateralization in feeding events quite evenly throughout the year. A total of 187 video recording sessions were made, on average 3.7 per week, each lasting on average 73 minutes (maximum duration of recording were limited by battery capacity) with the shortest session lasting 19 minutes and the longest 95 minutes. Before the first video recording started, the setup had been placed inside the aviary for 7 days for habituation. This setup was placed 1.40 m from the closest, and 5.14 m from the furthest feeders and perches in the feeding area, so that birds would not be influenced by the presence of others nearby, and at a height of 0.88 m, close to the height of the remaining feeders (which were either 1.20 m or 1.55 m), from which these waxbills were habituated to feed.

I observed all video-recordings following a protocol to identify feeding events and score lateralization. Every time a waxbill landed on the setup, it would normally 1) land in the perch, 2) approach the feeder, and 3) feed from the feeder. In line with this pattern of behaviour, each time that a bird landed on the perch I noted the time of landing in the video and whether the bird landed with its right or left side towards the food (hereafter, *landing side*). Next, I registered the *feeding side*, whether the bird fed with its left or right side of the body towards the food (**Figure 1**).

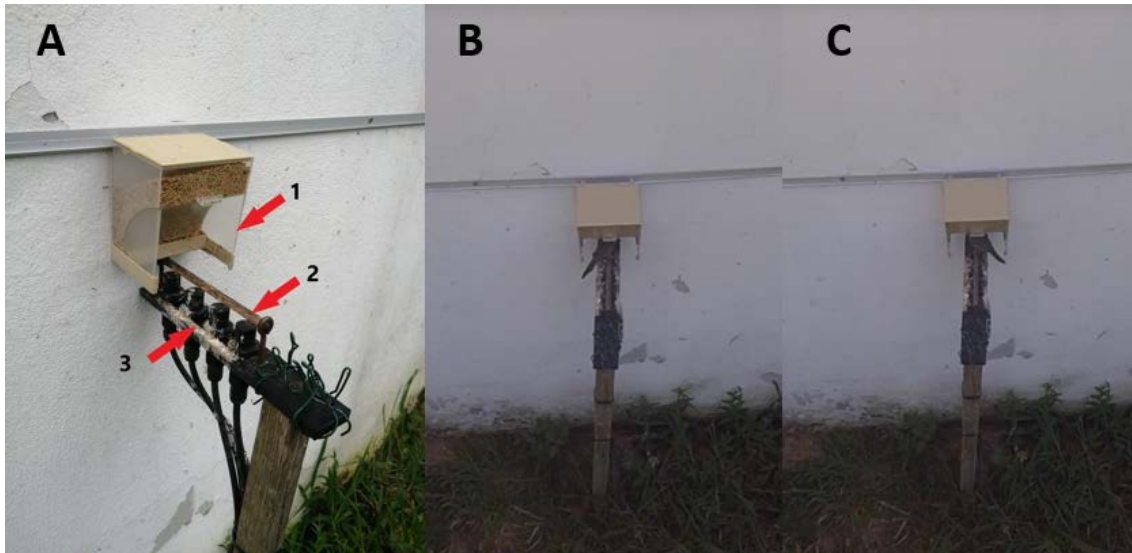


Figure 1: A) Experimental setup with 1) the adapted feeder, 2) perch, 3) four RFID antennae under the perch. B) A waxbill feeding from its left side and C) another feeding from its right side.

If an individual approached the feeder or fed whilst using both sides alternately, I noted those events as “both sides”, rather than as “left” or “right”. For an observation of a feeding event to be considered undisturbed and valid, the bird had to approach the food and feed for a minimum duration of 10 seconds, during which time no other bird landed on the apparatus. I used 10 seconds as threshold because previous information from the RFID system showed that the vast majority of feeding events lasted at least that amount of time (see *Figure 2* in Beltrão et al., 2021). If a bird was disturbed by others during any of these two steps (*landing* and *feeding*), or if it abandoned the feeder before 10 seconds, the feeding event was not considered for quantifying laterality. If a bird arrived at the feeder while another was already there and had to wait for the other bird to leave or actively displaced it before feeding, this event would also not be considered a valid observation, due to possible interference with behaviour. When a bird arrived at the feeder and another left simultaneously, without any contact between the two, the observation of the arriving bird could be a valid observation. To avoid misidentifying the arriving bird, because of slight time asynchronies between the RFID data stream and the video, I noted these events so that the ID of the departing bird was not considered when identifying the arriving bird.

To evaluate whether the *feeding side* can be interpreted as a behavioural preference, as opposed to simply be a consequence of the side that the bird came from and landed, I used 1647 feeding events video-recorded during the entire period of data collection (from May 12th 2021 to May 31st 2022) to calculate the proportion of times

that the *landing* and *feeding sides* were the same or differed. These 1647 cases corresponded to all valid feeding events in which waxbills fed exclusively using only one side of the body (left or right). *Landing side* and *feeding side* were the same in 40% of instances and were different in 60% of instances. *Landing side* thus did not predict *feeding side*, and the observed proportion approached a random 50-50%. Therefore, *feeding side* in this setup should reflect a choice of side by the bird, not constrained by from which side the bird came to the perch, and can be used as an assay of behavioural lateralization.

The individual identity (ID) of the video-recorded birds was retrieved from the RFID data stream of the four antennae under the perch perpendicular to the adapted feeder. For this, I registered the day and time (in hours and minutes) at which each video-session started (in a video log file) and, at the beginning of each video, I passed a distinct PIT tag by these antennae, so as to synchronize the RFID data stream with the video recording. Individuals observed in video recordings were identified using information from the RFID data stream, using a custom-written algorithm in *R* (version 4.2.1; R Core Team 2022). Briefly, for each video-session the algorithm first searches the RFID data stream for the code from the PIT tag that I passed in the beginning of the video, to match the time in the video and in the data stream. Then, for each valid video observation of a feeding event, it searches for identifications of waxbills in the RFID data stream using a backward time buffer of 5 seconds relative to the time noted in the video log, and a forward time buffer corresponding to the time interval between the focal bird and the next one landing on the system (up to a limit of 60 seconds). These backward time buffers were used because slight time asynchronies were possible between the video and RFID data. The forward time buffer was used because the focal bird might not be read by the RFID antennae immediately after landing. If a feeding event had been noted as starting by the time another bird was departing (without any contact between the two), then the script would discard readings with the ID of the departing bird in order to identify the arriving bird. Identification of a bird's identity was considered valid when only readings of one ID were detected within the buffer ranges. If multiple IDs were detected within the buffer range, or no ID was detected by the RFID system, then a single ID could not be attributed to that feeding event and would not be analysed further.

For each individual waxbill with 10 or more valid feeding events ($n = 22$ individuals), I computed a "Lateralization Index" (LI) and an "Absolute Lateralization Index" (ALI). I used data from individuals with 10 or more observations to compute robust indexes of lateralization. LI was calculated as $LI = (R-L)/(R+L)$, where R and L are the number of

times the bird fed from its right or left side, respectively. LI varies between 1 for birds always feeding with their right side, to -1 for birds always feeding with the left side and indicates the *direction* of lateralization. ALI was calculated as the absolute value of LI (ALI = |LI|), and varies between 0, when an individual feeds with its left or right side an equal number of times, to 1, if an individual always uses the same side. This index quantifies the *strength* of lateralization (irrespective of the direction adopted by individuals). These indexes of lateralization (LI and ALI), for each individual, are available in the supplementary material.

2.3. Individual-, population- and species-level lateralization

To test if individual waxbills differed in behavioural lateralization from each other, I performed a χ^2 test, comparing counts of feeding events for either direction across individuals with more than 10 valid feeding observations ($n = 22$ individuals). This tests whether waxbill individuals differ statistically in the proportion of times they used either side of the body to feed. To test for population-level lateralization, I performed a one-sample *Wilcoxon test* for non-normal variables, using the values of “Lateralization Index” (LI) calculated for individuals with more than 10 valid feeding observations ($n = 22$ individuals). This tests the null hypothesis that the mean LI does not differ from 0; if significant, it indicates that the majority of individuals are lateralized towards the same direction (either left or right) when feeding.

To assess species-level lateralization, beyond my study population, I collected 200 photographs of common waxbills from around the world feeding on seeds or perching on near-vertical grass (*Poacea*) stems, published in the citizen-science website *eBird* (www.eBird.org). I included photographs perching on near-vertical grass stems because waxbills in nature mostly eat grass seeds by perching at the top of the stem, both in Africa (Goodwin, 1982) and their different invasive ranges worldwide (Cardoso & Reino, 2018; Da Silvaxs & Oren, 1990; Oren & Smith, 1981; Sullivan et al., 2015). The protocol to select photographs and quantify lateralization consisted first in searching for “Common waxbill” in the eBird website, then following the list of results and downloading photographs of waxbills feeding from grass seeds or perching on near-vertical ($>45^\circ$) grass stems, so that it was clear on which side of the bird the grass inflorescence or seeds would be located. Only one photograph taken by the same photographer was downloaded to avoid quantifying lateralization twice for the same waxbill. Before quantification of behavioural lateralization, I randomly selected half of

the photographs and mirrored the image, so as to avoid unconscious or conscious bias on the part of a second person scoring lateralization, blindly to which photographs had or had not been flipped. The person scoring lateralization evaluated each photograph by observing if the bird clearly had one side towards the food while eating or, when perching, had one side towards the top of the stem. For photographs of waxbills feeding, if food was located on top, or on both sides of the bird simultaneously, the photo was classified as “ambiguous” and was not used for further analysis. For photos with more than one waxbill perching, if the same number of birds were seen perched in contrary directions the photo was also considered ambiguous; otherwise, a “majority rule” was applied and the most utilized direction to perch was noted. In the end a total of 41 photos were classified as ambiguous, resulting in a sample of 159 photographs. After reversing the scores for the photographs that had been flipped, I compared the number of times that waxbills used either side of the body towards the direction of the seeds.

2.4. Relations with phenotypic differences and social dominance

To test if phenotypic differences among individuals, such as differences in cognition, personality and stress levels were associated with differences in behavioural lateralization, I used the phenotypic data available in Gomes et al. (2020), who studied the same waxbill individuals as in this work, and to which I also refer for detailed methods. Here I briefly describe each phenotype.

As described in Gomes et al. (2020), the sex of each individual was assessed molecularly from a small blood sample ($< 100 \mu\text{L}$), and a measure of *body size* was computed as the first principal component (PC1) from a Principal Component Analysis (PCA) on two skeletal measurements: tarsus length and head-plus-bill length (PC1 in this work explained 71% of variation in the data and had high loadings for both morphological measures: 0.84).

Mirror tests have been shown to reliably assay personality type along a reactive-proactive axis in common waxbills (Carvalho et al., 2013; Funghi et al., 2018; Gomes et al., 2020; Guerra et al., 2020), and I therefore used behavioural scores of mirror tests to assay personality. Briefly, mirror tests took place in a small cage with a mirror that was covered during the first 5 minutes of the test and exposed in the last 5 minutes. During the period with the mirror exposed, the time birds spent looking in the direction

of the mirror, the number of vocalizations, changes of position within the cage and fast movements were quantified from video recordings. A PCA on these behaviours resulted in a PC1 whose high values indicate a more proactive response (more vocalizations, changes of position in the cage and fast movements), while low values indicated a more reactive response (more attention towards the mirror). Mirror tests were conducted twice for each bird, separated by a time period of 6 weeks. Since mirror tests have been shown to be repeatable in this species (Carvalho et al., 2013; Funghi et al., 2018; Gomes et al., 2020; Guerra et al., 2020), the averaged value of PC across the two mirror tests was used.

Breath rates were obtained by counting belly movements from video-recordings, during the first 5 minutes of the mirror test described above, while the mirror was covered (Gomes et al., 2020). The counts of belly movements were then curated using an algorithm that corrects for missing or duplicated counts (details in Gomes et al., 2020), and mean breath rate computed from these data. Again, I used the mean value of the two times each waxbill was filmed.

The *tonic immobility* assays consisted in placing each waxbill on its back and measuring the time in seconds that it would stay still (i.e., in a state of tonic immobility) before flying away, for a maximum of 60 seconds. Tonic immobility may be an index of fear (Gallup, 1979; Pusch et al., 2018), and in the common waxbill is not related with personality differences along their reactive-to-proactive axis (Carvalho et al., 2013; Funghi et al., 2018; Gomes et al., 2020; Guerra et al., 2020). Tonic immobility test was made immediately after each mirror test, and I again used the average scores on the two assays.

As a test of inhibitory control, which is a proxy for cognitive ability, I used data from a *detour-reaching task*. This involved habituation and training phases, where waxbills learned to detour an opaque cylinder with open ends to obtain seeds inside, and then a test phase, consisting of 15 trials presenting seeds inside a transparent (rather than opaque) cylinder. *Detour-reaching performance* was computed as the proportion of valid trials that were successful (i.e., seeds taken from the cylinder without first pecking its walls), as opposed to pecking or hitting the transparent wall, which indicate lack of inhibitory control to get the seeds behind the cylinder walls (Gomes et al., 2020).

I also assessed social dominance, following methods in Beltrão et al. (2021), who studied dominance hierarchies in this waxbill mesocosm population, and to which I refer for detailed methods. Briefly, I used the algorithm in Beltrão et al. (2021) to automatically identify aggressive displacements at feeders from the data stream of

RFID antennae at the feeders during the period of this study (specifically, data from every Sunday from May 12th 2021 to May 31st 2022, because there were never video recordings or other disturbances in the mesocosm on Sundays). The algorithm identifies displacements when the interval between an individual leaving a feeder and another arriving is less than 2 seconds, and both individuals stay at least 3 seconds in that feeder. Beltrão et al. (2021) showed, using video recordings, that these criteria identify aggressive displacements accurately. I then used these data, to compute Randomized *Elo-ratings*, a metric of position within social hierarchies (Sánchez-Tójar et al., 2018). In order to measure the quality of sampling effort, I also computed the ratio of interactions to individuals (i.e., number of interactions between dyads divided by the number of individuals) which was 16.70 (ideally it should be above 10), indicating that the inferred dominance hierarchy was reliably described (Sánchez-Tojar et al., 2018).

Before testing a possible relationship with behavioural lateralization, I evaluated if any strong correlations were present between any of the above phenotypes that could lead to multicollinearity issues. I computed Pearson correlation coefficients for all pairwise combinations of these phenotypic variables. Pairwise combinations involving tonic immobility used instead non-parametric Spearman correlation coefficients, since this variable strongly deviated from normality. Sample sizes differ in these correlations because of missing values in some phenotypes (**Table A1** in the *Attachments*). All pairwise correlation coefficients were lower than 0.35 in absolute value (**Table A1** in *Attachments*), which, by the absence of strong pairwise correlations (correlations > ca. 0.6), indicate that there are no multicollinearity issues.

To test which phenotypic traits were associated with individual differences in either the “Lateralization Index” (LI) or the “Absolute Lateralization Index” (ALI), I used generalized linear models (GLM) and a model selection approach weighting models by their Akaike’s information criterion (AICc; Hurvich & Tsai, 1989). I ran two separate GLM’s with either LI or ALI as the dependent variable, and as independent variables used the performance in the detour reaching task, scores from the mirror test, tonic immobility, social dominance, body size, breath rate and sex. The sample size for these two models was 13 individuals (5 females and 8 males). Interactions between independent variables were not included since there were no *a priori* predictions for those. AICc values were computed for models using all possible combinations of the independent variables. For model averaging I used all models within an interval of 6 AICc ($\Delta\text{AICc} < 6$) from the best model (i.e., the model with lowest AICc), weighing values by their Akaike weights. Prior to this analysis, all continuous variables were

standardized by subtracting the mean and dividing by 2x the standard deviation (Gelman, 2008), in order to make comparable model estimates for dichotomous predictors (sex) and continuous predictors (all others). For these models, all assumptions were checked graphically with the R package *performance* (version 0.9.1; Lüdtke et al., 2021). To perform model selection and model averaging, I used the R package “MuMIn” (version 1.46.0; Barton & Barton, 2015), with the function “dredge” for model selection, indicating that coefficient estimates were standardized by the standard deviation (argument “beta” = “sd”) and, for model averaging, the function “model.avg” (with argument “subset” = delta < 6). In these models, since there were missing values for some behavioural phenotypes, I used data from all waxbills with at least 5 valid behavioural observations.

2.5. Lateralization and social networks

To study the social network of waxbills in the mesocosm I used RFID data from all feeders and perches, from every Sunday during the year in which data on laterality were collected (May 12th 2021 to May 31st 2022). Following methods in Gomes et al. (2021), I used these data to infer proximity-based social networks. Briefly, social networks were computed after an optimization procedure that used different criteria to consider associations between birds, according to two categories: maximum distance at which birds were considered to be associated, and relaxing the synchrony of time between two subsequent birds in an antenna. Social networks build from these different criteria combinations were compared to identify the criteria that reveals a more robust social network. For each combination of these spatial-temporal criteria, social networks were computed and network structure was evaluated according to two robust descriptors of network structure (*coefficient of variation, CV, of strength of associations and Entropy*), since these have previously been demonstrated to be the ones that best reveal network structure (for details, see Gomes et al., 2021). The CV of strength of associations is a metric that measures the variation among strengths of associations between all pairs of individuals, and its value is higher when associations between different dyads are more heterogenous (i.e., more differentiated, indicating higher network structure). The metric of Entropy here used (*Shannon Entropy*) is a measure of how unpredictable animal associations in social networks are. In the case of these waxbills, high entropy indicates that associations between individuals are more unstable, and low entropy indicates more stable associations, which should result from

associations within each subgroup becoming stronger and more homogeneous, and thus leading to an increase in overall network structure (Gomes et al., 2021). According to these two descriptive statistics, the association criteria that revealed more structure in the social network was the synchronous time overlap between individuals at distances up to 40cm. For the social network, an *association index* was calculated for each possible pair of waxbills (i.e., dyads), as a measure of the strength of association between the two individuals. This association index is translated as the total time that any two individuals were together in the RFID system, divided by the amount of time that at least one of them was present in the system, and varied between 0 (if the dyad was never together) and 0.5 (if the dyad was always together). Significance of network structure was calculated against a null random model based on permutations (Gomes et al., 2021), generating 1.000.000 permutations and calculating null distributions for the metrics of CV and Entropy, and showed that the calculated network was significantly more structured than the null model ($p < 0.001$).

To test if individuals assorted or disassorted based on the direction (LI) or strength of lateralization (ALI), i.e., associated more or less with others similar to them, I used a procedure identical to Gomes et al. (2022). I first converted the values of either LI or ALI to similarity matrixes, by first standardizing each index, as recommended by Franks et al. (2021), and computing the absolute difference for each dyad of individuals. I then ran multimembership models (data from 26 waxbills; 12 females and 14 males), using MCMCglmm (Hadfield, 2010), with the social network (i.e., matrix of the strength of association between each dyad) as the dependent variable, and as predictors either of the lateralization similarity matrixes referred above. I also included the time each dyad spent in the RFID system, computed as the sum of the total time either individual in the dyad was in the RFID system, to control for a possible influence of sampling bias of individuals during data collection. As a random effect, I also included a multimembership grouping term (R function "mm"; Hadfield, 2010), which accounts for the ID of each bird in the dyads. Association strength values were log transformed as $\ln(x + 0.0001)$ to approach normality, and all continuous variables were standardized prior to analyses (subtracting the mean and dividing by the standard deviation; Gelman, 2008). For the MCMCglmm run of the multimembership model, I performed a total of 105.000 iterations, with a burn in of 5000, and thin of 10, to have a sample size of mostly 10.000. Trace plots and density of distributions were evaluated using MCMC samples, and autocorrelation between thinned samples was < 0.1 , meaning that correlation between random parameters was low.

To test if individual differences in the direction or strength of behavioural lateralization were associated with occupying more central or peripheral positions in the social network, I calculated the *weighted eigenvector centrality* for each individual. Higher values of weighted eigenvector centrality indicate that the focal individual has more and/or stronger associations with other individuals in the network, and is also weighted by the centrality of those associates (Farine & Whitehead, 2015). Weighted eigenvector centrality was calculated in R using the function “*evcent*” of the package “*sna*” (version 2.7; Butts, 2008), with default arguments and “*ignore.eval = FALSE*” to obtain the weighted metric. Then, following Gomes et al. (2022), I ran a general linear model (GLM) with eigenvector centrality as the dependent variable, and as predictors either the “Lateralization Index” or the “Absolute Lateralization Index”, separately (data from 26 waxbills). In addition, the total time each bird spent on the RFID system during the data collection was added as a predictor, so that I could control for possible biases of certain individuals being more present in the RFID system. For these models, all variables were standardized prior to analyses, as before, and model assumptions were checked with the R package *performance*.

3. Results

A total of 187 video-sessions were recorded, with durations ranging between 19 and 95 minutes. From these video recordings, I identified 3080 *feeding events*, of which 1756 were valid (i.e., the bird fed undisturbed for at least 10 seconds) for observing lateralization. Waxbills fed using their right-side in 1174 of those feeding events (67%), using their left-side in 473 occasions (27%), and using both sides alternately 109 times (6%). I was able to identify individuals from the RFID data in 884 of these 1756 valid feeding events, comprising a total of 34 different waxbills (see **Table A2** in the *Attachments*). Due to some inaccuracies in RFID antennae readings, not all valid feeding events were possible to be identified. Among those 34 individuals, the number of valid feeding observations ranged between 1 and 102. The number of waxbills with at least 10 valid behavioural observations, for use in the following analyses, was 22 (10 females and 11 males).

Among those 22 individuals, the “Lateralization Index” (LI) ranged between -0.92 (almost always feeding with the left side facing the seeds) and 1 (always feeding with the right side facing the seeds; **Figure 2**), and the “Absolute Lateralization Index” (ALI) ranged between 0.06 (almost no side preference) and 1 (always using the same side **Figure 3**). In addition, individual waxbills differed statistically in the proportion of times they used either side of the body to feed (χ^2 test: $\chi^2 = 329.4$; $df = 21$; $p < 0.001$). When testing for population-level lateralization, I found significant lateralization towards the right side of the body, with which most waxbills preferred to feed (one-sample Wilcoxon test: 95% CI: [0.16; 0.79]; $p < 0.01$; **Figure 2**).

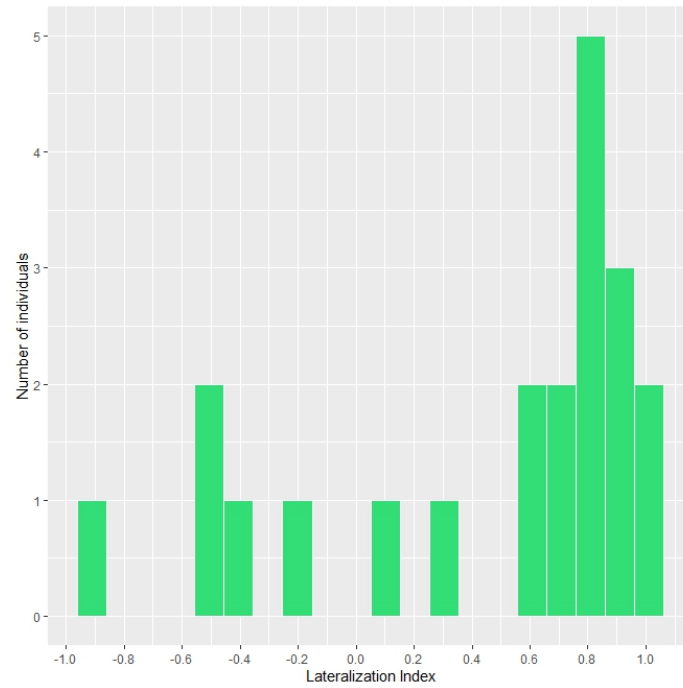


Figure 2: Histogram showing the distribution of the lateralization index (LI) for all individuals containing 10 or more valid feeding observations; LI can vary from -1 (always feeding from the left side) to 1 (always feeding from the right side).

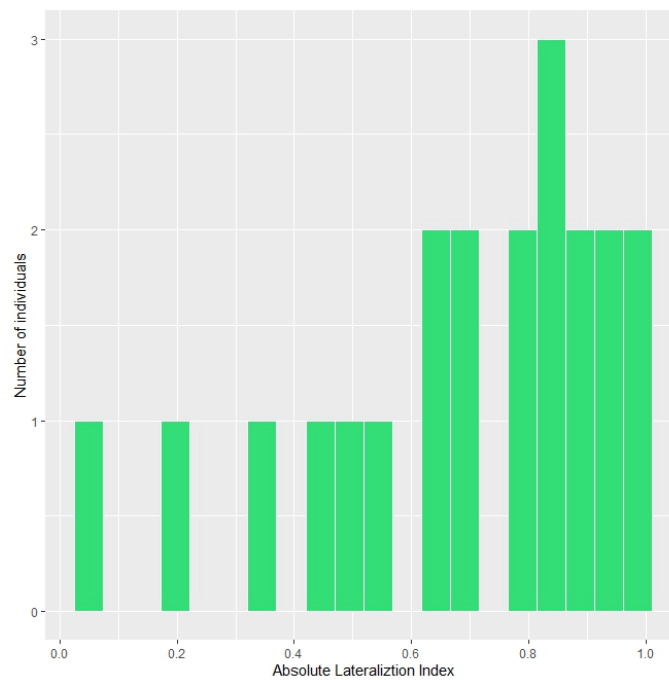


Figure 3: Histogram showing the distribution of strength of lateralization index (SL) for all individuals containing 10 or more valid feeding observations; SL can vary from 0 (feeding an equal number of times from each side) to 1 (always feeding from the same side).

However, analyses of photographs from around the world in the citizen-science website *eBird* showed an almost equal proportion of waxbills perching or feeding with either side facing the direction of the grass seeds or inflorescence: out of 154 photos where this direction could be determined, 76 had the left side and 78 the right side towards the seeds or tip of the grass.

3.1. Lateralization and Phenotypes

The best model relating phenotypes and social dominance to LI included only two predictors: performance in the detour-reaching task and sex (first line of **Table 1**), with no other models present within 2 ΔAICc from this. Model averaging of the 13 models within 6 ΔAICc from the best (**Table 1**) also showed that left-biased individuals performed better in the inhibitory control task (effect of inhibitory control: model averaged $\beta_{\text{st}} = -0.656$, $\text{SE} = 0.326$, $P = 0.057$, 95% CI = [-1.265; -0.221]), and females were on average more right-biased than males (effect of sex: model averaged $\beta_{\text{st}} = -0.483$, $\text{SE} = 0.356$, $P = 0.191$, 95% CI = [-1.159; -0.192]; all other effects: model averaged $|\beta_{\text{st}}| < 0.15$, $P > 0.65$; **Table 2**). The scatterplot in **Figure 4** shows LI and detour-reaching performance, and suggests sex differences in the relationship between these phenotypes: while right-lateralized males appear worse in detour-reaching performance than left-lateralized males, in females, who on average are more right-biased, this trend appears much weaker (**Figure 4**). To assess this, I ran a single GLM of LI using only detour-reaching and sex as predictors, and including their interaction, which confirmed that the interaction between sex and performance in the detour-reaching task was indeed significant ($\beta_{\text{st}} = -0.679$, $\text{SE} = 0.344$, $P = 0.080$, 95% CI = [-1.353; -0.005]; full model results in **Table 3**).

Table 1: Model selection approach for the performed GLM with "Lateralization Index" as dependent variable, showing all models within 6 AIC's; It is possible to observe the best model containing the Detour-reaching performance and Sex as predictors.

Body size	Change in breath rate	Dominance score	Detour-reaching performance	Mean breath rate	Mirror test	Sex	Tonic Immobility	AICc	Delta AICc	Akaike weight
			x			x		13.95	0	0.36
x	x		x					17.35	3.40	0.07
x			x			x		17.47	3.52	0.06
x			x					18.10	4.15	0.05
	x				x			18.32	4.37	0.04
	x		x			x		18.68	4.74	0.03
			x			x	x	19.05	5.10	0.03
					x			19.07	5.12	0.03
			x	x		x		19.44	5.49	0.02
		x	x			x		19.49	5.54	0.02
			x		x	x		19.52	5.57	0.02
								19.61	5.66	0.02
			x					19.86	5.91	0.02

Table 2: Model averaging approach for the relation between "Lateralization Index" and the analysed predictors, showing the standardized coefficients (β_{st}), standard errors (SE), p-values (P) and 95% confidence intervals.

MODEL AVERAGING

	β_{st} (SE; P)	95% CI
DETOUR-REACHING PERFORMANCE	-0.66 (0.33; 0.06)	(-1.26; -0.22)
SEX	-0.48 (0.36; 0.19)	(-1.16; -0.19)
BODY SIZE	-0.13 (0.28; 0.66)	(-1.26; 0.10)
CHANGE IN BREATH RATE	-0.07 (0.18; 0.71)	(-0.91; 0.13)
MIRROR TEST	-0.05 (0.18; 0.79)	(-1.14; 0.30)
TONIC IMMOBILITY	-0.01 (0.04; 0.93)	(-0.57; 0.34)
MEAN BREATH RATE	-0.001 (0.04; 0.97)	(-0.56; 0.46)
SOCIAL DOMINANCE	0.0009 (0.04; 0.98)	(-0.50; 0.57)

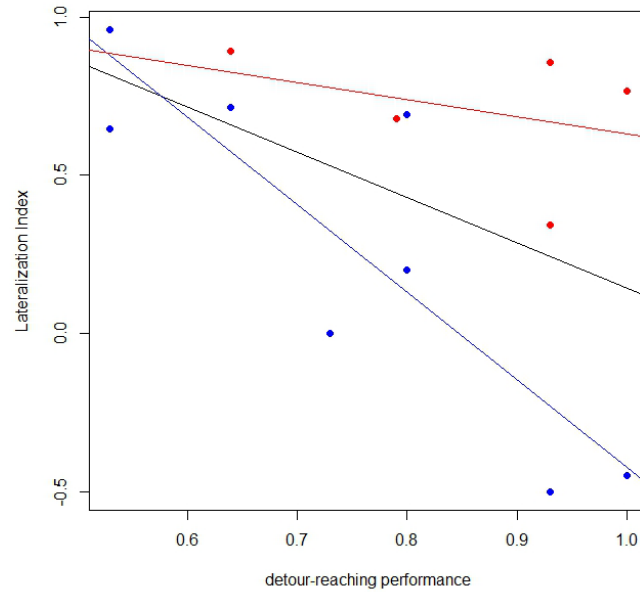


Figure 4: Scatterplot illustrating the relation between detour-reaching performance and the lateralization index in each sex: red for females (n = 5), and blue for males (n = 8); The grey regression line translates the relationship between performance in the detour-reaching task and the lateralization index for all individuals, while the red and blue regression lines represent the same relationship for females and males, respectively.

Table 3: Results from the GLM performed with detour-reaching performance, sex, and their interaction, as predictors of the “Lateralization Index” (LI); showed are the standardized coefficients (β_{st}), standard errors (SE), p-values (P) and 95% confidence intervals (95%CI).

SINGLE MODEL		
	β_{st} (SE; P)	95% CI
DETOUR-REACHING PERFORMANCE	-0.13 (0.29; 0.66)	(-0.70; 0.44)
SEX	-0.53 (0.16; 0.009)	(-0.83; -0.22)
DETOUR-REACHING PERFORMANCE*SEX	-0.68 (0.34; 0.08)	(-1.35; -0.004)

For the “Absolute Lateralization Index” (ALI), the model with lowest value of AICc was the null model (**Table 4**), which means that I cannot exclude the null hypothesis that the strength of lateralization is not related to the studied predictors.

Table 4: Model selection approach for the performed GLM with “Absolute lateralization Index” as dependent variable, showing all models within 2 AIC’s; It is possible to observe the best model containing no predictors.

Body size	Change in breath rate	Dominance Score	Detour-reaching performance	Mean breath rate	Mirror test	Sex	Tonic Immobility	AICc	Delta AICc	Akaike weight
								22.48	0	0.13
				x				23.41	0.93	0.08
					x			23.82	1.34	0.07
						x		24.33	1.85	0.05

3.2. Lateralization and social network

Waxbills did not assort nor disassort significantly by their “lateralization index” ($\beta_{st} = 0.10$, 95% CI = [-0.02; 0.21], $P_{MCMC} = 0.09$; total time in RFID: $\beta_{st} = 0.24$, 95% CI = [0.05; 0.43], $P_{MCMC} = 0.02$), meaning that individuals did not associate preferentially with others based on similarly or dissimilarity in feeding side lateralization. Similarly, there was no significant (dis)assortment based on the “absolute lateralization index” ($\beta_{st} = 0.05$, 95% CI = [-0.06; 0.16]; $P_{MCMC} = 0.37$; total time in RFID: $\beta_{st} = 0.24$, 95% CI = [0.05; 0.44], $P_{MCMC} = 0.02$).

The eigenvector centrality of individuals in the social network was not significantly predicted by their “lateralization index” ($\beta_{st} = 0.09$, SE = 0.17, P = 0.60; total time in RFID: $\beta_{st} = 0.61$, SE = 0.17, P = 0.001) nor by their “absolute lateralization index” ($\beta_{st} = -0.03$, SE = 0.17, P = 0.87; total time in RFID: $\beta_{st} = 0.60$, SE = 0.17, P = 0.002), indicating that neither the direction or the strength of lateralization was associated with more central or peripheral individual positions in the social network.

4. Discussion

Studying a wild-caught population in a large open-air mesocosm, I found that common waxbills show behavioural lateralization when feeding, with most individuals consistently feeding with the right side of their body towards the food, resulting in a population-level right-side behavioural lateralization. Nonetheless, I also found significant differences between individuals, both in the direction and strength of behavioural lateralization. On average, females were more right-side lateralized than males, who showed greater among-individual differences in lateralization. Individual differences in the direction of lateralization were correlated with performance in a detour-reaching task (a cognitive assay of inhibitory control; Gomes et al., 2020) with less right-lateralized males, but not females, having better inhibitory control ability. Finally, despite some theoretical predictions and results from other species, I did not find relationships between the direction or strength of behavioural lateralization and either morphology, personality, behavioural cues of stress, social dominance, social network centrality or patterns of association in the social network. I discuss these results in turn.

4.1. Population-level lateralization

When feeding from the experimental apparatus (i.e., a horizontal perch perpendicular to an isolated feeder on a wall), the majority of waxbills in this population preferentially adopted a right-side position, thus demonstrating population-level lateralization. In this context, the position individuals assume may reflect a preferential use of the right eye to view the seeds (Franklin III & Lima, 2001). Preferential use of the right eye during feeding has been described in multiple species (Canning et al., 2011; Robins et al., 2005; Robins & Rogers, 2004), including birds (Güntürkün et al., 2000; Mench & Andrew, 1986; Valenti et al., 2003). Simultaneously, since in my research setup the feeder is placed on a wall, having the right side towards the food could mean that waxbills choose to use the left eye for facing the surroundings to look for predators or monitor conspecifics (Franklin III & Lima 2001; Vallortigara & Rogers 2005). Specialization of the left eye for vigilance behaviour and predator detection is also

present in amphibians, mammals, birds and reptiles (see Dharmaretnam & Rogers, 2005; Lippolis et al., 2002; Lippolis et al., 2005; Martín et al., 2010). This similar pattern of hemisphere specialization across many species suggests that behavioural lateralization is an innate and ancestral trait, shared by a wide diversity of taxa (Ghirlanda & Vallortigara, 2004). As hypothesized by Vallortigara (2006), it is possible that in early solitary chordates, brain lateralization first appeared in order to increase brain efficiency and then, with the evolution of more complex sociality, social interactions favoured the alignment of the direction of behavioural lateralization across individuals. In line with this explanation, there is evidence of genetically determined direction of lateralization (e.g., in zebrafish; see Gunturkun & Ocklenburg, 2017). Thus, it is possible that the preferential direction adopted in my study population, is the result of ancient selective forces that determined a common pattern of lateralization that is maintained to this day. However, there are known exceptions to this directional pattern of lateralization with closely related species being lateralized in opposite directions (e.g., Bisazza et al., 1997; Franklin III & Lima, 2001).

As an alternative hypothesis, it could be that the population-level lateralization encountered here is the result of functional adaptation due to gregariousness in waxbills. Fitness advantages of lateralization at the population-level are thought to include better coordinated collective behaviour (Rogers, 2021). For example, shoaling fish may have an advantage from population-level lateralization since fish staying in large groups and escaping together in the same direction have lower probabilities of being predated – this is called the “dilution effect” (Vallortigara, 2006). Here I am not suggesting that feeding from the same side *per se* has collective advantages in the common waxbill. But perhaps feeding from the same side is symptomatic of population-level lateralization in other behaviours where group coordination is beneficial (Vallortigara & Rogers, 2005).

4.2. Species-level lateralization

When I looked into a more general hypothesis of species-level behavioural lateralization, by analysing photographs of waxbills feeding or perching in vertical grass stems from around the globe, I found no evidence for species-level lateralization. Waxbills were seen perching and feeding to either side an almost identical number of times. This could be due to several factors. 1) In the mesocosm, the feeding task that I studied does not correspond exactly to how waxbills feed in nature. For example, here

waxbills perched on a horizontal perch perpendicular to the feeder, while feeding in the wild usually implies using vertical or near-vertical perches (grass stems), which may cause more muscular fatigue on one side of the body and, thus, encourage alternation of sides. Also, here there was a wall blocking the view on the side of the feeder, so that, if waxbills prefer to use the left eye for vigilance, they will place their left side away from the food, while in nature vigilance is necessary towards both sides. Thus, ecological conditions, which here are different from the wild, could explain my result. Environmental conditions are known to affect behavioural lateralization in other species. For example, fish populations from high predation habitats are more strongly lateralized to use the right-eye when viewing a live predator than individuals from low predation sites (C Brown et al., 2004), and handedness in chimpanzees (*Pan troglodytes*) also varies with the region (Lonsdorf & Hopkins, 2005). 2) Considering that the photographs analysed were from waxbills belonging to different populations around the world, absence of apparent lateralization at the species level does not mean that individual populations are not lateralized. If the direction of population-level lateralization is not an ancestrally inherited trait, but instead results from within-population coordination of behaviour, then one population could have a preference for perching using the right side, and another for using the left.

4.3. Among-individual variation in the Common Waxbill

Despite the majority of waxbills in the study population showing right-side lateralization while feeding, individuals significantly differed from each other in the strength and direction of this lateralization. It has been shown in a wide range of taxa, including many bird species, that conspecific individuals differ in lateralization scores (Rogers & Kaplan, 2019). Such among-individual variation has been found to be associated with various individual behavioural characteristics, depending on the species, such as personality traits (Reddon & Hurd, 2009) and physiological stress (Byrnes et al., 2016), or with the ecological context (e.g. different predation pressures; C Brown et al., 2004) and genetic factors (Rogers, 2021). In the specific case of the waxbill population here studied, a minority of waxbills appeared not to be lateralized or were lateralized in the opposite direction of the majority, showing a left-side bias. The presence of non-lateralized and “inverse” individuals across populations, might result from adaptation to heterogeneous selection pressures or frequent changes in environmental conditions (Rogers & Vallortigara, 2015). It is also possible that frequency-dependent selection

explains that a minority of individuals within populations are lateralized differently (Vallortigara & Rogers, 2005). Frequency-dependant selection states that the minority individuals have a fitness advantage over the majority, as long as their number does not increase too much (Vallortigara & Rogers, 2005). For example, left-handedness in humans is thought to be maintained by this mechanism, and studies have shown that left handers may have an advantage in competition contexts, such as sports, since they are more unpredictable (Raymond et al., 1996). Left biased common waxbills could have disadvantages including, among others, not being as coordinated with the group, but have advantages in other tasks. This hypothesis is discussed in the next section.

4.4. Association between lateralization and inhibitory control

I found a significant association between the direction of behavioural lateralization and performance in an inhibitory control task (the detour-reaching task), where more left lateralized individuals, especially males, inhibited more accurately fruitless pecking at the transparent walls of a cylinder containing seeds inside (Gomes et al., 2020). This association was not due to individual differences in the strength of lateralization, but specifically differences along the left-side to right-side continuum. In other species, the most common finding is that more lateralized individuals, irrespective of the direction, are better at a wide variety of behaviours (Brown & Magat, 2011; McGrew & Marchant, 1997; Mench & Andrew, 1986). Similarly to my result, however, a recent study working with the zebrafish (*Danio rerio*) found a relationship between the direction of lateralization in a social task and scores from an inhibitory control test: zebrafish who preferred to use the right eye to view themselves in a mirror were better at suppressing attack towards live prey presented inside a transparent tube (Lucon-Xiccato et al., 2020). Despite the type of behavioural lateralization studied in zebrafish (looking at mirror image) and here in waxbills (feeding) being different, results from both these studies indicate that the direction of behavioural lateralization, rather than the degree of lateralization, can also be associated with individual differences in cognition. Furthermore, my result with waxbills suggests one potential advantage associated with being left side lateralized in this feeding task, improved ability of inhibitory control, which may help explain the persistence of some individuals in the population that are lateralized differently in relation to the majority.

4.5. Association between lateralization and sex

The two sexes demonstrated significant differences in the direction of lateralization. Females were all right biased ($LI \gg 0$), with the exception of one individual, and in males greater among-individual variation was evident, with left and right biased individuals being present. Sex differences in behavioural lateralization are not uncommon and have previously been reported in several species. For example, male dogs (*Canis familiaris*) preferentially used the left paw to remove an adhesive from the snout, while females preferred to use the right paw (Quaranta et al., 2004). Male zebrafish (*Danio rerio*) used the left eye more than females when observing a mirror image of themselves or, especially, a real opponent (Ariyomo & Watt, 2013). In the presence of a conspecific of the same sex, female poeciliid fish (*Gambusia hoolbroki* and *Girardinus falcatus*) show a strong right turning bias while males exhibit no turning preference (Bisazza et al., 1998). Bianki and Filippova (2003) highlighted the importance of considering sex in the study of cerebral lateralization and behavioural asymmetries, because the two sexes differ in terms of brain organization, possibly affecting observable animal behavioural lateralization. Furthermore, different behavioural characteristics and different life-history strategies of males and females could influence the selection of diverse patterns of behavioural lateralization between the two sexes (Reddon & Hurd, 2008; Ariyomo & Watt, 2013). It is important to take into consideration that the left-biased male waxbills here present belong to the minority group in a right-biased population and as suggested earlier, this minority could be maintained through frequency-dependent selection. For example, male poeciliid fish (*Girardinus falcatus*) show a preferred side to attempt copulation with females and given that the latter sex may be vigilant of unwanted copulatory attempts towards the majority side, the minority males that attempt to copulate in a way that females do not expect might have an advantage (Vallortigara & Bisazza, 2002; Vallortigara & Rogers, 2005). Alternative mating strategies could help explain the observed variation within males, since rare mating polymorphisms are thought to also be maintained by frequency-dependent selection within populations (Shuster, 2010). It could also be hypothesized that different competition styles from left-biased males would justify their presence in the population, as it has been theorized that minority individuals have some kind of advantage in contexts of competition. But, not supporting this hypothesis, I did not find an association between social dominance and behavioural lateralization.

5. Conclusion

Apart from the sex difference in lateralization and the association with detour-reaching performance, I found no further associations of behavioural lateralization and individual differences in other phenotypes: body size, personality type, cues of stress, social dominance, or centrality in the social network. Also, I found no effect of the strength or direction of behavioural lateralization on how individuals assorted or disassorted in their social network. These results contrast with studies in some other species, where behavioural lateralization has been associated with, for example, boldness (Found & St Clair, 2017; Reddon & Hurd, 2009), fearfulness (Rogers, 2009), stress (Byrnes et al., 2016; Ocklenburg et al., 2016), or social structure and stable dominance rankings (Rogers & Workman, 1989). Despite this, similarly to my results, studies in several other species did not encounter an association of behavioural lateralization with these phenotypes or aspects of social organization (Byrnes et al., 2016; Díaz González, 2021). Perhaps by studying other aspects of behavioural lateralization, or using larger sample sizes, some of these associations could be demonstrated in waxbills. My results, however, indicate that sex and cognition (here, inhibitory control, as assayed by a detour-reaching task) are the traits most strongly associated with behavioural lateralization.

The prominent association between behavioural lateralization and a cognitive trait in waxbills, amongst the diverse phenotypes that I also studied, support the theory that behavioural lateralization is the result of specialization of the cerebral hemispheres, with implications for cognition (Frasnelli & Vallortigara, 2018). In this respect, a highlight of my results, along with few others (e.g., Bibost & Brown, 2014; Lucon-Xiccato et al., 2020), is to show that the direction of behavioural lateralization, rather than its strength, may reflect cognitive differences among individuals. To my knowledge these results are also the first indicating that the association between behavioural lateralization and cognition can depend on sex, which might be related with the fact that male waxbills differed more in behavioural lateralization than females.

6. Bibliography

- Alonso, Y. (1998). Lateralization of visual guided behaviour during feeding in zebra finches (*Taeniopygia guttata*). *Behavioural Processes*, 43(3), 257-263.
- Anfora, G., Frasnelli, E., Maccagnani, B., Rogers, L. J., & Vallortigara, G. (2010). Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. *Behav Brain Res*, 206, 236-239.
- Ariyomo, T. O., & Watt, P. J. (2013). Aggression and sex differences in lateralization in the zebrafish. *Animal Behaviour*, 86, 617-622.
- Barton, K., & Barton, M. K. (2015). Package 'mumin'. *Version*, 1, 439.
- Beltrão, P., Marques, C. I., Cardoso, G. C., & Gomes, A. C. R. (2021). Plumage colour saturation predicts long-term, cross-seasonal social dominance in a mutually ornamented bird. *Animal Behaviour*, 182, 239-250.
- Berg, M. L., Micallef, S. A., Eastwood, J. R., Ribot, R. F. H., & Bennett, A. T. D. (2020). Spatial and temporal patterns of lateralization in a parrot species complex. *Evolutionary Ecology*, 34, 789-802.
- Bianki, V. L. v., & Filippova, E. B. (2003). *Sex differences in lateralization in the animal brain*. CRC Press.
- Bibost, A.-L., & Brown, C. (2014). Laterality influences cognitive performance in rainbowfish *Melanotaenia duboulayi*. *Animal cognition*, 17, 1045-1051.
- Bisazza, A., Facchin, L., Pignatti, R., & Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behavioural Brain Research*, 91, 157-164.
- Bisazza, A., Pignatti, R., & Vallortigara, G. (1997). Laterality in detour behaviour: interspecific variation in poeciliid fish. *Animal Behaviour*, 54, 1273-1281.
- Brown, C., & Bibost, A.-L. (2014). Laterality is linked to personality in the black-lined rainbowfish, *Melanotaenia nigrans*. *Behavioral Ecology and Sociobiology*, 68, 999-1005.
- Brown, C., & Braithwaite, V. A. (2005). Effects of predation pressure on the cognitive ability of the poeciliid *Brachyraphis episcopi*. *Behavioral Ecology*, 16, 482-487.
- Brown, C., Gardner, C., & Braithwaite, V. A. (2004). Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*. *Proc Biol Sci*, 271 Suppl 6, S455-457.

- Brown, C., & Magat, M. (2011). The evolution of lateralized foot use in parrots: a phylogenetic approach. *Behavioral Ecology*, *22*, 1201-1208.
- Butts, C. T. (2008). Social network analysis with sna. *Journal of statistical software*, *24*, 1-51.
- Byrnes, E. E., Pouca, C. V., Chambers, S. L., & Brown, C. (2016). Into the wild: developing field tests to examine the link between elasmobranch personality and laterality. *Behaviour*, *153*, 1777-1793.
- Byrnes, E. E., Vila Pouca, C., & Brown, C. (2016). Laterality strength is linked to stress reactivity in Port Jackson sharks (*Heterodontus portusjacksoni*). *Behav Brain Res*, *305*, 239-246.
- Canning, C., Crain, D., Eaton Jr, T. S., Nuessly, K., Friedlaender, A., Hurst, T., Parks, S., Ware, C., Wiley, D., & Weinrich, M. (2011). Population-level lateralized feeding behaviour in North Atlantic humpback whales, *Megaptera novaeangliae*. *Animal Behaviour*, *82*, 901-909.
- Cardoso, G. C., & Reino, L. (2018). Ecologically benign invasions: The invasion and adaptation of common waxbills (*Estrilda astrild*) in Iberia. In *Histories of Bioinvasions in the Mediterranean* (pp. 149-169). Springer.
- Carvalho, C. F., Leitão, A. V., Funghi, C., Batalha, H. R., Reis, S., Mota, P. G., Lopes, R. J., & Cardoso, G. C. (2013). Personality traits are related to ecology across a biological invasion. *Behavioral Ecology*, *24*, 1081-1091.
- Chivers, D. P., McCormick, M. I., Warren, D. T., Allan, B. J. M., Ramasamy, R. A., Arvizu, B. K., Glue, M., & Ferrari, M. C. O. (2017). Competitive superiority versus predation savvy: the two sides of behavioural lateralization. *Animal Behaviour*, *130*, 9-15.
- Da Silvaxs, J. M. C., & Oren, D. C. (1990). Introduced and invading birds in Belém, Brazil. *The Wilson Bulletin*, 309-313.
- Dadda, M., Koolhaas, W. H., & Domenici, P. (2010). Behavioural asymmetry affects escape performance in a teleost fish. *Biol Lett*, *6*, 414-417.
- Dadda, M., Zandona, E., Agrillo, C., & Bisazza, A. (2009). The costs of hemispheric specialization in a fish. *Proc Biol Sci*, *276*, 4399-4407.
- Daisley, J. N., Mascalzoni, E., Rosa-Salva, O., Rugani, R., & Regolin, L. (2009). Lateralization of social cognition in the domestic chicken (*Gallus gallus*). *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*, 965-981.
- Denenberg, V. H. (1981). Hemispheric laterality in animals and the effects of early experience. *Behavioral and Brain Sciences*, *4*, 1-21.

- Dharmaretnam, M., & Rogers, L. (2005). Hemispheric specialization and dual processing in strongly versus weakly lateralized chicks. *Behavioural Brain Research*, *162*, 62-70.
- Díaz González, S. (2021). Laterality in Chimpanzees: Links with Behavioural Style and Social Networks.
- eBird. (2021). eBird: An online database of bird distribution and abundance [www.eBird.org]. eBird, Cornell Lab of Ornithology, Ithaca, New York. Available: <http://www.ebird.org>. (Accessed: Date [April, 2021])
- Farine, D. R., & Whitehead, H. (2015). Constructing, conducting and interpreting animal social network analysis. *Journal of Animal Ecology*, *84*, 1144-1163.
- Found, R., & St Clair, C. C. (2017). Ambidextrous ungulates have more flexible behaviour, bolder personalities and migrate less. *R Soc Open Sci*, *4*, 160958.
- Franklin III, W. E., & Lima, S. L. (2001). Laterality in avian vigilance: do sparrows have a favourite eye? *Animal Behaviour*, *62*, 879-885.
- Franks, D. W., Weiss, M. N., Silk, M. J., Perryman, R. J., & Croft, D. P. (2021). Calculating effect sizes in animal social network analysis. *Methods in Ecology and Evolution*, *12*, 33-41.
- Frasnelli, E. (2013). Brain and behavioral lateralization in invertebrates. *Front Psychol*, *4*, 939.
- Frasnelli, E., & Vallortigara, G. (2018). Individual-Level and Population-Level Lateralization: Two Sides of the Same Coin. *Symmetry*, *10*.
- Frasnelli, E., Vallortigara, G., & Rogers, L. J. (2011). Origins of brain asymmetry: lateralization of odour memory recall in primitive Australian stingless bees. *Behav Brain Res*, *224*, 121-127.
- Freire, R., van Dort, S., & Rogers, L. J. (2006). Pre- and post-hatching effects of corticosterone treatment on behavior of the domestic chick. *Horm Behav*, *49*, 157-165.
- Funghi, C., Trigo, S., Gomes, A. C. R., Soares, M. C., & Cardoso, G. C. (2018). Release from ecological constraint erases sex difference in social ornamentation. *Behavioral Ecology and Sociobiology*, *72*, 1-12.
- Gallup, G. G. (1979). Tonic immobility as a measure of fear in domestic fowl. *Animal Behaviour*.
- Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in medicine*, *27*, 2865-2873.

- Ghirlanda, S., Frasnelli, E., & Vallortigara, G. (2009). Intraspecific competition and coordination in the evolution of lateralization. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, 861-866.
- Ghirlanda, S., & Vallortigara, G. (2004). The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proc Biol Sci*, 271, 853-857.
- Giljov, A., & Karenina, K. (2019). Differential roles of the right and left brain hemispheres in the social interactions of a free-ranging ungulate. *Behav Processes*, 168, 103959.
- Gomes, A. C. R., Beltrão, P., Boogert, N. J., Cardoso, G. C., & Ridley, A. (2022). Familiarity, dominance, sex and season shape common waxbill social networks. *Behavioral Ecology*, 33, 526-540.
- Gomes, A. C. R., Boogert, N. J., & Cardoso, G. C. (2021). Network structure and the optimization of proximity-based association criteria. *Methods in Ecology and Evolution*, 12, 88-100.
- Gomes, A. C. R., Guerra, S., Silva, P. A., Marques, C. I., Trigo, S., Boogert, N. J., & Cardoso, G. C. (2020). Proactive common waxbills make fewer mistakes in a cognitive assay, the detour-reaching task. *Behavioral Ecology and Sociobiology*, 74.
- Goodwin, D. (1982). *Estrildid finches of the world*. British Museum (Natural History).
- Goursot, C., Dupjan, S., Kanitz, E., Tuchscherer, A., Puppe, B., & Leliveld, L. M. C. (2019). Assessing animal individuality: links between personality and laterality in pigs. *Curr Zool*, 65, 541-551.
- Guerra, S., Gomes, Ana Cristina R., & Cardoso, Gonçalo C. (2020). Long-term consistency despite cross-seasonal changes in personality traits of common waxbills. *Behaviour*, 157, 781-806.
- Güntürkün, O., Diekamp, B., Manns, M., Nottelmann, F., Prior, H., Schwarz, A., & Skiba, M. (2000). Asymmetry pays: visual lateralization improves discrimination success in pigeons. *Current Biology*, 10, 1079-1081.
- Gunturkun, O., & Ocklenburg, S. (2017). Ontogenesis of Lateralization. *Neuron*, 94, 249-263.
- Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of statistical software*, 33, 1-22.
- Harris, A., & Davis, J. (1993). Finches and sparrows: an identification guide. In: London: Christopher Helm.
- Hurvich, C. M., & Tsai, C.-L. (1989). Regression and time series model selection in small samples. *Biometrika*, 76, 297-307.

- Isparta, S., Salgirli Demirbas, Y., Bars, Z., Cinar Kul, B., Gunturkun, O., Ocklenburg, S., & Da Graca Pereira, G. (2020). The relationship between problem-solving ability and laterality in cats. *Behav Brain Res*, 391, 112691.
- Lippolis, G., Bisazza, A., Rogers, L. J., & Vallortigara, G. (2002). Lateralisation of predator avoidance responses in three species of toads. *Laterality: Asymmetries of Body, Brain and Cognition*, 7, 163-183.
- Lippolis, G., Westman, W., McAllan, B., & Rogers, L. (2005). Lateralisation of escape responses in the stripe-faced dunnart, *Sminthopsis macroura* (Dasyuridae: Marsupialia). *Laterality: Asymmetries of Body, Brain, and Cognition*, 10, 457-470.
- Lonsdorf, E. V., & Hopkins, W. D. (2005). Wild chimpanzees show population-level handedness for tool use. *Proceedings of the National Academy of Sciences*, 102, 12634-12638.
- Lucon-Xiccato, T., Chivers, D. P., Mitchell, M. D., & Ferrari, M. C. O. (2017). Prenatal exposure to predation affects predator recognition learning via lateralization plasticity. *Behavioral Ecology*, 28, 253-259.
- Lucon-Xiccato, T., Montalbano, G., Dadda, M., & Bertolucci, C. (2020). Lateralization correlates with individual differences in inhibitory control in zebrafish. *Biol Lett*, 16, 20200296.
- Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, 6.
- Magat, M., & Brown, C. (2009). Laterality enhances cognition in Australian parrots. *Proc Biol Sci*, 276, 4155-4162.
- Martín, J., Lopez, P., Bonati, B., & Csermely, D. (2010). Lateralization when monitoring predators in the wild: a left eye control in the common wall lizard (*Podarcis muralis*). *Ethology*, 116, 1226-1233.
- McGrew, W. C., & Marchant, L. F. (1997). On the other hand: current issues in and meta-analysis of the behavioral laterality of hand function in nonhuman primates. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, 104, 201-232.
- Mench, J., & Andrew, R. (1986). Lateralization of a food search task in the domestic chick. *Behavioral and neural biology*, 46, 107-114.
- Morgante, M., Ganesella, M., Stelletta, C., Versace, E., Cannizzo, C., Ravarotto, L., & Vallortigara, G. (2007). Short-term adaptive response in strongly versus weakly lateralized dairy ewes. *Italian Journal of Animal Science*, 6, 567-569.
- Ocklenburg, S., Korte, S. M., Peterburs, J., Wolf, O. T., & Gunturkun, O. (2016). Stress and laterality - The comparative perspective. *Physiol Behav*, 164, 321-329.

- Oren, D. C., & Smith, N. J. (1981). Notes on the status of the Common African Waxbill in Amazonia. *The Wilson Bulletin*, 281-282.
- Payne, R., & del Hoyo, J. (2010). Family Estrildidae (Waxbills). *Handbook of the Birds of the World*, 15, 234-377.
- Piddington, T., & Rogers, L. J. (2013). Strength of hand preference and dual task performance by common marmosets. *Anim Cogn*, 16, 127-135.
- Pikalík, M., Pipová, N., Majláthová, V., Connors, V. A., & Majláth, I. (2021). Lateralization at the individual and population levels of European green lizard in Slovak Karst. *acta ethologica*, 25, 15-24.
- Pusch, E. A., Bentz, A. B., Becker, D. J., & Navara, K. J. (2018). Behavioral phenotype predicts physiological responses to chronic stress in proactive and reactive birds. *General and Comparative Endocrinology*, 255, 71-77.
- Quaranta, A., Siniscalchi, M., Frate, A., & Vallortigara, G. (2004). Paw preference in dogs: relations between lateralised behaviour and immunity. *Behavioural Brain Research*, 153, 521-525.
- R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Raymond, M., Pontier, D., Dufour, A.-B., & Møller, A. P. (1996). Frequency-dependent maintenance of left handedness in humans. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263, 1627-1633.
- Reddon, A. R., & Hurd, P. L. (2009). Individual differences in cerebral lateralization are associated with shy–bold variation in the convict cichlid. *Animal Behaviour*, 77, 189-193.
- Robins, A., Chen, P., Beazley, L. D., & Dunlop, S. A. (2005). Lateralized predatory responses in the ornate dragon lizard (*Ctenophorus ornatus*). *NeuroReport*, 16, 849-852.
- Robins, A., & Rogers, L. J. (2004). Lateralized prey-catching responses in the cane toad, *Bufo marinus*: analysis of complex visual stimuli. *Animal Behaviour*, 68, 767-775.
- Roche, D. G., Amcoff, M., Morgan, R., Sundin, J., Andreassen, A. H., Finnøen, M. H., Lawrence, M. J., Henderson, E., Norin, T., Speers-Roesch, B., Brown, C., Clark, T. D., Bshary, R., Leung, B., Jutfelt, F., & Binning, S. A. (2020). Behavioural lateralization in a detour test is not repeatable in fishes. *Animal Behaviour*, 167, 55-64.
- Rogers, & Kaplan. (2019). Does Functional Lateralization in Birds Have any Implications for Their Welfare? *Symmetry*, 11.

- Rogers, L., & Vallortigara, G. (2015). When and Why Did Brains Break Symmetry? *Symmetry*, 7, 2181-2194.
- Rogers, L., & Workman, L. (1989). Light exposure during incubation affects competitive behaviour in domestic chicks. *Applied Animal Behaviour Science*, 23, 187-198.
- Rogers, L., Zappia, J., & Bullock, S. (1985). Testosterone and eye-brain asymmetry for copulation in chickens. *Experientia*, 41, 1447-1449.
- Rogers, L. J. (2009). Hand and paw preferences in relation to the lateralized brain. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364, 943-954.
- Rogers, L. J. (2021). Brain Lateralization and Cognitive Capacity. *Animals (Basel)*, 11.
- Sánchez-Tójar, A., Schroeder, J., & Farine, D. R. (2018). A practical guide for inferring reliable dominance hierarchies and estimating their uncertainty. *Journal of Animal Ecology*, 87, 594-608.
- Schiffner, I., & Srinivasan, M. V. (2013). Behavioural lateralization in Budgerigars varies with the task and the individual. *PLoS One*, 8, e82670.
- Schnell, A. K., Jozet-Alves, C., Hall, K. C., Radday, L., & Hanlon, R. T. (2019). Fighting and mating success in giant Australian cuttlefish is influenced by behavioural lateralization. *Proc Biol Sci*, 286, 20182507.
- Shuster, S. M. (2010). Alternative mating strategies. *Evolutionary behavioral ecology*, 434-450.
- Siniscalchi, M., D'Ingeo, S., & Quaranta, A. (2017). Lateralized Functions in the Dog Brain. *Symmetry*, 9.
- Sullivan, M. J., Davies, R. G., Mossman, H. L., & Franco, A. M. (2015). An anthropogenic habitat facilitates the establishment of non-native birds by providing underexploited resources. *PLoS One*, 10, e0135833.
- Templeton, J. J., Mountjoy, D. J., Pryke, S. R., & Griffith, S. C. (2012). In the eye of the beholder: visual mate choice lateralization in a polymorphic songbird. *Biol Lett*, 8, 924-927.
- Valenti, A., Anna Sovrano, V., Zucca, P., & Vallortigara, G. (2003). Visual lateralisation in quails (*Coturnix coturnix*). *Laterality: Asymmetries of Body, Brain and Cognition*, 8, 67-78.
- Vallortigara, G. (2006). The evolutionary psychology of left and right: costs and benefits of lateralization. *Dev Psychobiol*, 48, 418-427.
- Vallortigara, G., & Bisazza, A. (2002). How ancient is brain lateralization. *Comparative vertebrate lateralization*, 9-69.

Vallortigara, G., & Rogers, L. (2005). Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behavioral and Brain Sciences*.

Whiteside, M. A., Bess, M. M., Frasnelli, E., Beardsworth, C. E., Langley, E. J. G., van Horik, J. O., & Madden, J. R. (2018). Low survival of strongly footed pheasants may explain constraints on lateralization. *Sci Rep*, 8, 13791.

7. Attachments

Table A1: Correlations between measured predictors.

	Detour-reaching performance	Mirror Test	Tonic Immobility ^α	Mean breath rate	Change in breath rate	Body Size
	$r(P)$; N = 32	$r(P)$; N = 38	$r(P)$; N = 38	$r(P)$; N = 38	$r(P)$; N = 38	$r(P)$; N = 66
Mirror Test	0.35 (0.049) *					
Tonic Immobility ^α	0.098 (0.59)	0.186 (0.26)				
Mean breath rate	0.248 (0.17)	0.27 (0.097)	-0.05 (0.75)			
Change in breath rate	0.05 (0.77)	0.08 (0.627)	-0.26 (0.11)	0.016 (0.92)		
Body Size	-0.345 (0.052)	-0.085 (0.61)	0.09 (0.58)	-0.246 (0.136)	-0.11 (0.51)	
Dominance score	-0.219 (0.227)	0.057 (0.729)	0.227 (0.169)	-0.273 (0.096)	-0.238 (0.148)	0.17 (0.168)

^α: Correlations performed for Tonic Immobility were non-parametric (Spearman Correlations);

*Significant values ($P < 0.05$)

Table A2: List of all waxbills (ID) that I was able to identify in the RFID system, containing the number of times that each utilized its right, left, or both sides to feed from my setup during each feeding event; The total number of events where individuals were identified is also presented, along with the calculated values of “lateralization index” (LI) and “Absolute lateralization index” (ALI).

ID	Right	Left	Both	Total Events	LI	ALI
0007272CC2	1	0	0	1	1	1
0007996AA8	1	0	0	1	1	1
0007274349	2	0	0	2	1	1
00077005F3	3	0	0	3	1	1
000769BC10	3	1	0	4	0.500	0.500
00079A9B29	2	2	0	4	0	0
00077177E5	3	0	1	4	1	1
00076996A0	5	0	0	5	1	1
00072719D2	3	2	1	6	0.200	0.200
00072726EC	3	3	1	7	0	0
0007271573	6	1	0	7	0.714	0.714
00079AE557	9	0	0	9	1	1
000726E8A0	2	7	1	10	-0.556	0.556
000768BB48	10	0	0	10	1	1
00072716E5	13	1	0	14	0.857	0.857
00071E2012	13	1	0	14	0.857	0.857
00072709A6	14	3	0	17	0.647	0.647
00072737E1	13	3	1	17	0.625	0.625
0007272E33	21	4	0	25	0.680	0.680
0007272924	22	4	3	29	0.692	0.692
0007997526	27	3	0	30	0.800	0.800
00079AEDFA	32	0	0	32	1	1
000727049A	8	24	0	32	-0.500	0.500
00072739EA	17	15	4	36	0.063	0.063
00072727A7	35	2	0	37	0.892	0.892
0007695429	21	14	2	37	0.200	0.200
000727162E	38	5	2	45	0.767	0.767
00072708E8	48	1	0	49	0.959	0.959
000726F440	2	49	0	51	-0.922	0.922
0007271045	48	4	3	55	0.846	0.846
000727110D	19	28	9	56	-0.191	0.191
000726FE34	52	3	1	56	0.891	0.891
0007271A8D	47	23	7	77	0.343	0.343
0007271408	24	63	15	102	-0.448	0.448