Cognitive and neural correlates of innovation in wild finches

Jean-Nicolas Audet

Department of Biology,

McGill University, Montréal

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Table of Contents

List of Figures

APPENDIX III

Abstract

Behavioral innovations are defined as novel solutions to problems encountered by animals in the wild. Innovativeness can facilitate survival in new or changing environments. Anthropization is a major perturbation that is expected to favor innovative animals. While innovation rate is known to vary geographically and with relative brain size in vertebrates, the environmental and molecular bases of innovation are largely unknown. The work presented in this thesis aims at elucidating some of the ultimate and proximate causes of innovation, specifically 1) how innovations appear in the wild and how urbanization influences them 2) what are the neuromolecular bases of innovation. We characterized innovation in the Barbados bullfinch *Loxigilla barbadensis*, a tame and innovative bird species. First, we found that innovations can spread among individuals in the field, but they also appear independently. We also showed that innovative problem-solving skills positively vary with the gradient of urbanization, along with boldness, neophobia and immunocompetence. To elucidate the neural bases of innovativeness, we compared *L. barbadensis* with its sister species in Barbados, the black-faced grassquit *Tiaris bicolor*. Both species are territorial and overlap in their habitat use, but are highly divergent in their foraging strategies, *T. bicolor* being shy and conservative. Following a battery of tests in captivity, we found that the two species differed sharply in their problem-solving skills and risk-taking behaviors, but were similar for all other measured traits. Molecular analyses revealed that genes related to synaptic activity were particularly differentially expressed between the species in the associative pallium (mesopallium, nidopallium). Genes related to neurogenesis and neuron signaling were more expressed in *L. barbadensis* and those differences were more pronounced in the nidopallium caudolaterale (NCL), a region thought to be functionally analogous to the mammalian prefrontal cortex. At a finer scale, we identified two groups of neurotransmitter receptors that were remarkably divergent: NMDA and metabotropic glutamate receptors. In particular, the GRIN2B:GRIN2A ratio differed, which is in accordance with the literature on mammalian cognition. Our results suggest precise molecular targets for a potential case of convergent evolution of cognition in birds and mammals.

Résumé

Les innovations comportementales sont définies comme étant de nouvelles solutions à des problèmes rencontrés par les animaux en nature. L'anthropisation est une cause de perturbation majeure qui est censée favoriser les animaux innovateurs. Au niveau proximal, l'innovation devrait être contrôlée par des propriétés neurales intrinsèques spécifiques. Toutefois, bien qu'il soit établi que le taux d'innovation varie géographiquement et en fonction et de la taille relative du cerveau chez les vertébrés, les bases environnementales et moléculaires de l'innovation sont largement inconnues. Les travaux présentés dans cette thèse visent à élucider certaines causes ultimes et proximales de l'innovation, à savoir 1) comment les innovations apparaissent en nature et comment l'urbanisation affecte l'innovation et 2) quelles sont les bases neuromoléculaires de l'innovation. Nous avons caractérisé l'innovation chez le sporophile de la Barbade *Loxigilla barbadensis*, une espèce d'oiseau innovatrice et facile à approcher. Premièrement, nous avons trouvé que les innovations peuvent se diffuser entre individus, mais aussi apparaître de façon indépendante. Nous avons également découvert que la capacité de résolution de problèmes variait positivement avec le gradient d'urbanisation, en même temps que la témérité, la néophobie et l'immunocompétence. Pour élucider les bases neurales de l'innovation, nous avons comparé *L. barbadensis* avec son espèce-sœur à la Barbade le sporophile cici *T. bicolor*. Les espèces sont toutes deux territoriales et se recoupent sur le terrain, mais sont hautement divergentes dans leurs stratégies d'alimentation, *T. bicolor* étant timide et conservateur. Suivant une batterie de tests comportementaux en captivité, nous avons montré que les deux espèces différaient fondamentalement dans leur capacité à résoudre des problèmes et leur comportement de prise de risque, mais qu'elles étaient similaires pour tous les autres traits mesurés. Nos analyses moléculaires ont révélé que les gènes reliés à l'activité synaptique étaient particulièrement différentiellement exprimés entre les deux espèces dans le pallium associatif (mésopallium et nidopallium). Les gènes associés à la neurogenèse et la signalisation synaptique étaient plus exprimés chez *L. barbadensis* et ces différences se sont avérées plus prononcées dans le nidopallium caudolaterale (NCL), une région considérée comme étant fonctionnellement analogue au cortex préfrontal chez les mammifères. À une échelle encore plus fine, nous avons

identifié deux groupes de récepteurs de neurotransmetteurs qui étaient remarquablement divergents : les récepteurs de glutamate NMDA et métabotropiques. En particulier, le ratio GRIN2B:GRIN2A différerait entre les deux espèces, ce qui est en accord avec la littérature sur la cognition mammalienne. Nos résultats suggèrent des cibles moléculaires précises pour un cas potentiel d'évolution convergente de la cognition chez les oiseaux et les mammifères.

Acknowledgements

First of all, I am extremely grateful to my supervisor Louis Lefebvre for his immense dedication and his incredible trust in me. During my Ph.D., although I had all the freedom that I wished, Louis was always there for me when I needed him, regardless of his physical location in the world. He constantly did everything that he could to help improve my academic career in various aspects, even if this might mean setting aside his own. Louis is the kind of professor I wish to become: erudite, creative, devoted, an excellent teacher and a remarkable researcher but still a very approachable and enjoyable person. I would definitively not be the same if he had not been in my life.

I thank Simon Ducatez, an exceptional scientist that acted as a mentor during my PhD. Simon has taught me many, many things, and always with an extraordinary humility despite his vast and broad knowledge. Simon is probably the most generous person I encountered in my life, always ready to put his work on hold if he can be of assistance to someone else. I also thank Simon, the friend, for his pleasant company in Barbados, be it during these long days of mist-netting or during those crazy nights at the *Rum Shop*. Regardless of where in the world Simon ends up, I hope we will always be close, both as scientific collaborators as well as friends. I also thank Laure Cauchard for introducing me to Barbados and its adorable avian characters, for help with bird capture and for stimulating discussions. I also acknowledge the hard work of Lima Kayello for behavioral tests on bullfinches and grassquits during the first field season in Barbados, a work that needed a lot of patience and commitment.

I am particularly grateful to Erich Jarvis and Lauren O'Connell, who welcomed me in their labs to share their great expertise in *in situ* hybridization and transcriptome sequencing, respectively. Those essential collaborations that combined the strengths of some of the best researchers in the world have allowed me to achieve my very ambitious project. I also thank Jon Sakata and Ehab Abouheif of my supervisory committee for their helpful comments and suggestions.

Je remercie également ma femme Mélanie Couture, qui, dans un acte d'amour incommensurable, a accepté de me suivre et de m'appuyer dans cette aventure

insensée qu'est une carrière académique en biologie, avec tous les compromis que cela comporte. Son appui lors de mes nombreuses périodes de découragement, son réconfort avant mes terrorisantes présentations orales et ses efforts pour me fournir le meilleur environnement de travail qui soit auront été autant de facilitateurs pour le complètement de mon doctorat. Merci aussi à ma mère Carmen Elliott et mon beau-père Marc Hubert pour leur aide essentielle à plusieurs niveaux, incluant leur énorme assistance aux soins de leur petite-fille Alice, qui nous ont permis de respirer un peu. Finalement merci à mon frère François-René et mes amis, Mathieu et Gab, J-F et Léo, Laure et Thomas, Olivier et Geneviève, P-A et M-C, Peggy, Annie et Julie, pour leur soutien et toutes ces *frettes* qui m'ont permis de décompresser.

Preface and contribution of co-authors

This is a manuscript-based thesis. The manuscripts in Chapter 1, 2 and 3 have been published in peer-reviewed journals and the manuscript in Chapter 4 has been submitted to *Nature*. The Appendices I, II and III have also been published in peer-reviewed journals. I am the first author of all the articles presented in this thesis except for the manuscript presented in Appendix III on which I am the last author. All chapters and appendices represent **novel contributions to knowledge**, except appendix II which is an opinion piece. Chapter 1 shows for the first time that the spatial distribution of a feeding innovation observed in the field can have multiple independent origins; chapter 2 shows for the first time that differences in immunocompetence accompany differences in cognition associated with avian urbanization; chapter 3 adds two new species to the repertoire of birds that pass the string-pulling test; chapter 4 is the first study to document differences in gene expression in six brain areas of sister species of wild birds; appendix I is the first study to sex with molecular techniques the monomorphic Barbados bullfinch, while appendix III presents the first phylogenetic analysis of innovation rate differences in neotropical birds.

The manuscript in **Chapter 1** was published in *Animal Cognition* in 2013 (16:525–529). I co-first authored this work with Simon Ducatez. All the data gathering was done by myself and Simon. Louis Lefebvre helped Simon and me with the manuscript writing.

The manuscript in **Chapter 2** was published in *PLoS One* in 2016 (11:e0156112). Simon Ducatez and I conducted the experiments and statistical analyses together and wrote the manuscript with Louis Lefebvre.

The manuscript in **Chapter 3** was published in *Behavioral Ecology* in 2016 (27:637–644). The field work and analyses were conducted by myself and Simon Ducatez. The manuscript writing was done by myself, Louis Lefebvre and Simon Ducatez.

The manuscript in **Chapter 4** was submitted to *PNAS* in June 2017. This project required a large number of experimental techniques. I conducted the field work (bird capture and behavioral testing) with the aid of Lima Kayello, Simon Ducatez and Laure Cauchard. The results of the lid-flipping problem-solving task in this chapter have already been

included in the M. Sc. thesis of Lima Kayello, but never published because they were always intended as a part of a broader study that the *PNAS* paper brings together. Quantification of *in situ* hybridization and immunohistochemistry was done by Sara Perillo under my close supervision; I performed *in situ* hybridization with the help of Jason Howard and Erich Jarvis for the molecular biology (sequence analyses, cloning) and logistical aspects respectively. I prepared the DNA libraries for next-generation sequencing with guidance from Lauren O'Connell. I conducted all bioinformatics analyses alone and some statistical analyses with the assistance of Simon Ducatez. The manuscript was written by me, Louis Lefebvre, Simon Ducatez and Erich Jarvis.

The methodological article in **Appendix I** was published in *Zoological Science* in 2014 (31:687–691) following routine PCR testing that I was performing in the lab of Erich Jarvis. I conducted statistical analyses with Simon Ducatez and the manuscript was written be myself, Simon Ducatez and Louis Lefebvre.

The opinion piece in **Appendix II** was published in *Behavioral Ecology* in 2017 (advance access: arx007). This is the result of ideas that I had after taking a graduate seminar in the department of Psychology at McGill. Louis Lefebvre greatly contributed to the manuscript.

The article of **Appendix III** was an invited paper published in Philosophical Transactions of the Royal Society B (371: 20150188), part of the issue: "Innovation in animals and humans: understanding the origins and development of novel and creative behaviour". Simon Ducatez did the statistical analyses, Louis Lefebvre compiled all the innovations, Simon and I did the field work, and we wrote the article with Louis Lefebvre.

General Introduction

Some animals are opportunistic and innovative, and rapidly take advantage of new feeding opportunities that become available in their environment. Other animals are shy and conservative, and thus generally avoid situations where humans modify their habitat. These differences are particularly important in the current period of environmental changes, which have major effects on many organisms, some of which are going extinct while others are profiting via invasions and urbanization. Understanding the traits that affect this variation is important for both applied and theoretical reasons, as it can help mitigate the anthropogenic crisis as well as help us understand how evolution works in periods of intense environmental change.

In the 7 chapters and appendices of this thesis, I use a series of methods from behavioral ecology, comparative psychology and neuroscience to ask questions about the mechanisms and consequences of novel foraging behaviors in an innovative species.

Animal cognition in the wild

Manifestations of cognition in the wild

Cognition refers to a set of mechanisms by which animals process, store and act on information from the environment, including perception, learning, memory and decision-making (Shettleworth 2010). Japanese macaques that wash potatoes, British tits that open milk bottles, or Darwin's finches, chimpanzees and New-Caledonian crows that use tools are some of the most striking manifestations of cognition in the wild (Fisher and Hinde 1949; Kawamura 1954; Eibl-Eibesfeldt 1961; Hunt 1996). In some cases, the first instance of these cognitive examples is witnessed and reported by a human observer and is thus considered an innovation. In the first review paper on animal innovation, Kummer and Goodall (1985) defined it as a solution to a novel problem or a novel solution to an old one. Historically, the first innovation mapped in the field was the opening of milk bottles by blue tits and great tits, initially seen in 1921 in a small town in southwest England, Swaythling (Fisher and Hinde 1949). For decades, the major focus of research on innovation was the process by which they spread in animal

populations. Only recently has research shifted to asking why it was tits, chimpanzees and macaques that innovated the most famous cases of novel foraging techniques or what environmental, cognitive and neural processes co-vary with innovativeness.

Quantifying cognition in the wild

It is one thing to describe spectacular cases of cognition in the wild and another to devise quantitative, operational measures that allow broad comparative studies and detailed experiments on a large number of species. Lefebvre and colleagues (1997) found an elegant solution to this problem. They examined thousands of reports from ornithologists that have a tradition of systematically communicating unusual observations, often in specialized ornithology journals. Lefebvre et al. compiled all the mentions of "new", "first observed" or "unusual" behaviors in the ornithology literature, to build an innovation database. This information was used to obtain innovation rates at the species or higher taxonomic levels. This has been an extremely successful approach to compare cognition in a large number of wild species. During nearly 30 years, Lefebvre and colleagues complemented this database by incorporating potential biases such as research effort, phylogeny and geographical data. This ambitious research program has culminated in a reliable innovation database that can be used to test predictions with large scale comparative methods, like the relation between innovation and brain size (see Brain size as a correlate of innovation, below) (Lefebvre et al. 2004). Reader and Laland (2002) have shown that similar patterns occur in primates.

Ecological contexts that favor cognition

It is assumed that rapid changes in environmental conditions can drive the appearance of innovations (Kummer and Goodall 1985). Animal introductions to novel environments are extreme cases of such rapid changes. Such a high level of transformation in environmental contingencies presumably requires specific behavioral traits, and innovativeness is expected to be one of them. Sol et al. (2002, 2005) directly tested this hypothesis by assessing the relationship between the documented histories (successful or unsuccessful) of bird introductions and the innovation rate of introduced species in their region of origin. They found a robust positive association between invasion success, innovativeness and relative brain size (Sol et al. 2002; Sol et al. 2005).

This evidence highlights the importance of being able to solve problems to survive when unexpected environmental upheavals arise.

Islands are ecosystems that highlight evolutionary adaptations because of their young age, their relatively low species diversity and often unique assemblage of species (Losos and Ricklefs 2009), often favoring the appearance of innovative behaviors. The low predation rate together with the lower competition that characterize insular ecosystems are thought to stimulate innovativeness. For example, Darwin's finches in the Galápagos island display high levels of tameness and innovativeness, and some of them even use tools (Teschke et al. 2011; Tebbich et al. 2012; Teschke et al. 2013; Tebbich and Teschke 2014). The famous New-Caledonian crow *Corvus moneduloides*, an extraordinarily large-brained bird that manufactures and uses tools as well, also evolved on the islands of the New Caledonia archipelago (Hunt 1996; Cnotka et al. 2008). In the Cocos Islands, the finch *Pinaroloxias inornata* shows a broad array of individual specializations that are thought to be maintained via individual and social learning (Werner and Sherry 1987). Barbados shares similar insular properties with New Caledonia and the Galápagos and Cocos islands, with the additional feature that it has a high degree of anthropogenic disturbance. All those features greatly favor tame, opportunistic and innovative species like the Carib grackle (Overington et al. 2011) and the Barbados bullfinch *Loxigilla barbadensis*, the species on which I will focus for most of this thesis.

Urbanization is one of the most important sources of environmental disturbance for animals, and it is expected to favor certain behavioral traits, including innovation (Lowry et al. 2013; Sol et al. 2013). Urbanization can promote risk-taking behaviors through habituation to human proximity and the more frequent presence of novel situations. Several lines of evidence show that urbanization increases boldness in birds (Cooke 1980; Knight et al. 1987; Valcarcel and Fernández-Juricic 2009; Evans et al. 2010; Lowry et al. 2011; Atwell et al. 2012; Møller and Tryjanowski 2014). However, tests of neophobia, which measure the fear of novelty, yield contradictory results when comparing between urban and rural populations (increased neophobia: Echeverría and Vassallo 2008; Miranda et al. 2013, unchanged: Echeverría and Vassallo 2008; Bókony

et al. 2012; decreased: Sol et al. 2011; Tryjanowski et al. 2016). Anthropized environments are also predicted to favor innovative animals because of the increased novel feeding opportunities they provide. However, problem-solving abilities have rarely been compared between urban and rural animals. Sol and colleagues (2011) found that introduced common mynas *Acridotheres tristis* from urban environments solve technical problems faster than their suburban counterparts. Those results on the effect of urbanization on personality and problem-solving warrant further investigation to establish unequivocally which behaviors are favored or disadvantaged in perturbed environments.

Animal cognition in the lab

The psychological tradition

Researchers in the field of psychology have been interested in cognition for over a century. For that reason, some argue that classical psychology tasks should be implemented to investigate cognition in wild animals, instead of more ecologically-based tasks that were recently developed to measure cognitive abilities (Rowe and Healy 2014). It could in fact be tempting to borrow decades of knowledge and methods from the fields of cognitive and experimental psychology.

First, it is important to acknowledge that in psychology, the ultimate goal is to understand the human mind. For that aim, experimental psychologists use standardized paradigms often involving animals, mostly rodents, as simple models of human behaviors. Comparative psychologists that are interested in comparing cognition between taxa usually apply the same paradigms that were developed to measure human intelligence, but to a broader variety of taxa.

Reversal learning is undoubtedly the most widely used paradigm employed by psychologists to measure cognition. In its simplest form, the subject has to associate a reward with a stimulus in the presence of an unrewarded stimulus (acquisition learning phase). Then, both stimuli are switched, to reward the initially unrewarded stimulus and vice-versa. It is generally assumed that reversal learning scores reflect general

intelligence, *g*. In fact, reversal learning tasks were originally used to assess mental deficits in "feebleminded", "imbeciles", "idiots" and "low I.Q." individuals (Gardner 1945; Plenderleith 1956; O'Connor and Hermelin 1959). Later, psychologists began to use the term "flexibility" to characterize "intelligent" or "creative" behaviors (Adcock and Martin 1971). Today, the concept of "behavioral flexibility" is abundantly tackled in psychology, and it is measured by reversal learning, self-control and set-shifting tasks, among others. Recently, behavioral ecologists have borrowed the concept of "behavioral flexibility" to label cognition in wild animals, but they have added other, often unrelated, tasks to measure this ability. The danger here is that the term "behavioral flexibility" might refer to such a wide array of tasks and cognitive processes that it becomes meaningless (see discussion in Appendix II).

Psychology tasks never had the mandate of measuring any adaptive ability in non-human animals. To study adaptive variation in behavior, the experimental tasks that are employed need to be ecologically relevant, implying that they must reflect behavior used in the wild. There is no reason for an animal to perform well in a reversal learning task if the task requires an ability that is not useful in the wild, and that may have never existed in this particular animal. Consequently, it appears most popular tasks developed in psychology might not be useful for behavioral ecologists interested in behavior in the wild, as their relevance were never demonstrated for wild animals.

Behavioral ecology and neuroecology approaches

Contrary to psychologists who are mainly interested in proximal mechanisms of cognition, behavioral ecologists focus on both proximal and ultimate dimensions affecting behavior (Tinbergen 1963). Since the late 1980's, the behavioral ecology approach has been applied to cognition and given rise to the field of neuroecology. Neuroecology aims at investigating the adaptive variation in cognition and the brain (Sherry 2006). The ultimate objective of neuroecologists is to elucidate how natural selection acts on cognition and its neural mechanisms (Sherry 2006). This approach consists in i) observing adaptive behaviors in wild animals ii) experimentally measuring the cognitive skills behind those behaviors and iii) elucidating the neural bases of those behaviors.

This methodology was first successfully implemented to characterize food-caching behavior in chickadees and to elucidate the neural correlates of this behavior. The work of Sherry and colleagues, accomplished during the last three decades, has unequivocally shown that food-caching abilities can be measured experimentally using spatial learning tasks and that the hippocampus is the structure responsible for that behavior (reviewed in Sherry 2011).

In this thesis, the neuroecological approach will be applied to innovation. To eventually assess the neural bases of innovation, we first needed to experimentally assess it in controlled conditions. To accurately assess innovation, laboratory tasks need to represent the type of problems that are encountered in the wild. Accordingly, the most popular and accepted tasks to measure innovation are "obstacle-removal" type of problem-solving tasks, which were specifically designed to mimic innovations of this type (Griffin and Guez 2014) and are based on the earliest known innovation in birds, the removal and/or piercing of bottle tops by tits (Fisher and Hinde 1949).

Choosing a species that is not specialized in problem-solving to study innovation would be in contradiction with the logic of the neuroecological approach. For example, van Horik and Madden (2016) have assessed problem-solving in pheasant chicks (*Phasianus colchicus*), a species that is not particularly recognized for its problem-solving skills (*Phasianidae* are among the least innovative of all bird families: they have only 2 out of the 1030 reported technical innovations in the Overington et al., 2009 database). Correspondingly, the results of van Horik and Madden show that their birds solved in a random, non-repeatable manner the problems presented to them. van Horik and Madden (2016) concluded that problem-solving is not the result of cognition – although they did not measure any other cognitive trait. An alternative explanation is that pheasant chicks are unable to understand the problems that were presented to them (while some may have accidently solved the problems), making this species very poor problem-solvers, which is in accordance with their very low innovation score (Overington et al. 2009). Therefore, in this particular case, the observed variation in problem-solving score may not be caused by variation in a cognitive skill but rather by unrelated variables that increase the chance of randomly solving the problems.

Consequently, any further examination of a cause for the observed variation in futile. This is an excellent demonstration that choice of the model species is crucial to be able to adequately study adaptive specializations.

Brain correlates of cognition

Brain size

The innovation rate database allowed researchers to test for the first time for correlations of an ecologically valid measure of cognition with a variety of potentially associated variables in a large number of species. The most famous breakthrough resulting from this approach was the discovery of a robust correlation between innovativeness and relative brain size (Reader and Laland 2002; Sol et al. 2005; Overington et al. 2009). The finding that innovation rate is correlated with the size of specific brain structures responsible for complex functions, the neocortex or the pallium for example, is even more striking (Lefebvre et al. 1997; Timmermans et al. 2000; Lefebvre et al. 2002; Reader and Laland 2002). This approach, although convincing and effective, has often been criticized in light of the fact that simple volumetric measurements exclude the compartmentalization and the remarkable complexity of the brain, and that contemporary methods of molecular neurobiology should be used to better explain the molecular mechanisms responsible for differences in cognitive skills and innovativeness (Mace et al. 1980; Healy and Rowe 2007).

Mammalian cognition

The fields of experimental and cognitive psychology have made tremendous progress in the last century. Psychological investigations involving both humans and rodents have been essential for understanding mental illnesses and cognition in general. As a consequence, the organization of the mammalian brain is now very well deciphered. It is well established that the prefrontal cortex (PFC) is essential for several complex cognitive abilities. The skills that are measured by reversal learning, self-control and set-shifting tasks, designed under the umbrella term “behavioral flexibility” in psychology, are all controlled, at least in part, by the PFC (Dalley et al. 2004). However,

there is increasing evidence that suggests some compartmentalization and sub-structural specializations of the PFC. For instance, reversal learning requires an intact orbitofrontal cortex whereas set-shifting is thought to be controlled by the lateral prefrontal cortex in primates or medial prefrontal cortex in rats (Chudasama and Robbins 2006; Nilsson et al. 2015).

In birds, several lines of evidence, using different approaches and techniques (connectome: Shanahan et al. 2013; single-unit recording: Rose and Colombo 2005; Veit and Nieder 2013; Lengersdorf et al. 2015; receptor architecture: Rose et al. 2010; Herold et al. 2011; temporary inactivation: Helduser and Güntürkün 2012; lesions: Mogensen and Divac 1993) suggest that the caudolateral nidopallium (NCL) is the avian equivalent of the PFC. Most of this evidence has been gathered using standard psychology tasks. However, the neural correlates of problem-solving have rarely been investigated in mammals, therefore it is hard to predict if the NCL also controls problem-solving skills.

At the molecular level, we now know in great detail the involvement of neurotransmitter receptors in cognition. In vertebrates, the glutamate receptor family is one of the largest that has an excitatory function in learning and in the generation of complex behaviors (Abel and Lattal 2001; Zhao et al. 2005). Glutamate receptors are divided into three subfamilies of ionotropic receptors: AMPA, NMDA and kainate receptors, and a subfamily of metabotropic receptors coupled to G proteins (mGluRs) (**Figure 1**) (Nakanishi 1992; Dingledine et al. 1999; Attwell and Gibb 2005). When they are activated by glutamate, ionotropic receptors gate ion channels and metabotropic receptors change their conformation, in both cases resulting in the activation of second messengers (RNA) to ultimately activate target genes (Nakanishi 1992; Dingledine et al. 1999; Attwell and Gibb 2005). The GRIN2B (also called NR2B) subtype of NMDA receptors attracted much attention some years ago, and still today, for its role in cognition. Remarkably, overexpressing the gene encoding the GRIN2B subunit in transgenic mice (Tang et al. 1999) and rats (Wang et al. 2009) yielded improved learning and memory. Conversely, GRIN2B deficient mice were shown to have significant deficits in learning and memory (Brigman et al. 2010). Pharmacological upregulation

(Rammes et al. 2009; Xie et al. 2012) or downregulation (Dalton et al. 2011) of GRIN2B induces similar effects.

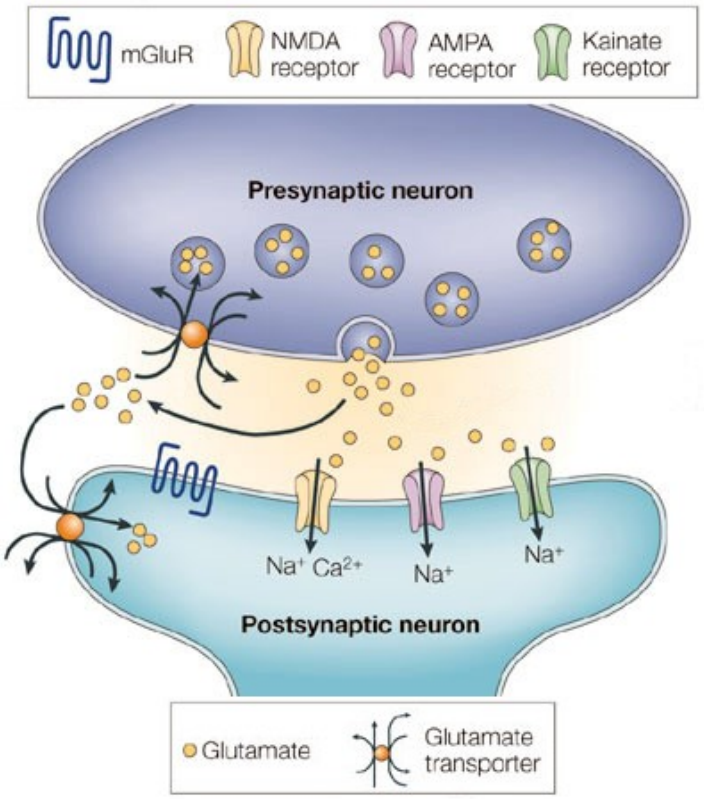

Figure 1. Neuronal glutamate receptors. Glutamate released from the presynaptic neuron acts on NMDA, AMPA, kainate and metabotropic (mGluR) receptors. (adapted from Attwell and Gibb 2005)

On the contrary, manipulating the expression of GRIN2A (NR2A) induces opposite effects (Marquardt et al. 2014). In fact, it was discovered later that it is actually the *ratio* of GRIN2B/GRIN2A expression that is responsible for variation in learning and memory. It was shown that the GRIN2B/GRIN2A ratio is positively associated with LTD and LTP efficiency, dendritic spine density and learning skills (Yashiro and Philpot 2008; Cui et al. 2013). The overexpression of another subtype of NMDA receptors, GRIN1 (NR1), increases learning and neurogenesis while knockdown of GRIN1 generates opposite effects (Kalev-Zylinska et al. 2009).

The latter discoveries have profoundly increased our knowledge of the neural bases of cognition in mammals. However, virtually no investigations have been conducted on the molecular underpinnings of ecologically-relevant behaviors like innovative problem-solving. In addition, most of the knowledge at this level of precision is on mammals and is lacking in other taxa.

Evolution of the avian brain

Among non-mammals, birds are probably the best candidates for investigations concerning the neural correlates of cognition. First, behavioral ecologists have a long tradition of behavioral field studies in birds. Several behavioral manifestations of their cognition are well-documented in nature (e.g. tool-use, innovation, food-caching, etc.) and ecologically-relevant tasks are increasingly being developed in captivity (e.g. problem-solving tasks to measure innovation, spatial learning to measure food caching). More importantly, thanks to the songbird vocal learning program conducted during the last four decades, we are starting to have a fairly good understanding of bird brain organization.

Birds and mammals are separated by more than 320 million years of independent evolution (Laurin and Reisz 1990; Laurin and Reisz 1996). Birds, along with other non-mammalian vertebrates, were long thought to lack the complex cognition of mammals, behaving mostly in a stereotyped "instinctive" manner and being incapable of learning. This profoundly influenced the view of early comparative neurobiologists, who considered the avian brain as being mostly constituted of basal ganglia, which are responsible for basic primary functions in humans (subpallium in **Figure 2A**) (Edinger 1896; Edinger 1908). However, with the behavioral data showing that birds can in fact display "complex" cognitive abilities, as well as recent molecular and anatomical data, this view is now obsolete, and accordingly, the avian brain nomenclature have been entirely revised (**Figure 2B**) (Reiner et al. 2004; Jarvis et al. 2005; Jarvis et al. 2013).

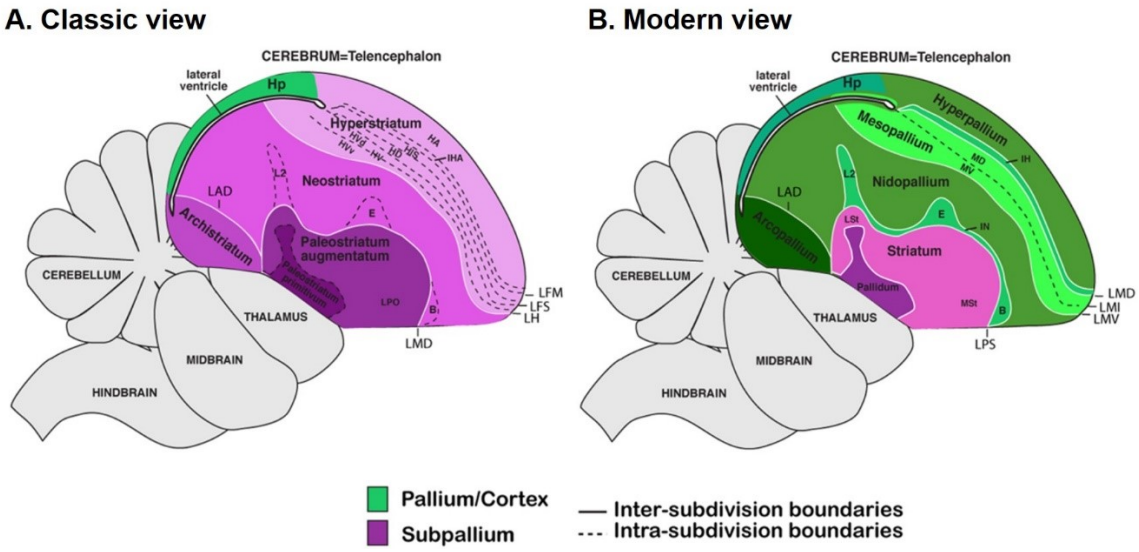

Figure 2. Avian brain subdivisions. A. Classic view, with most of the brain deriving from subpallium/striatal origins. **B.** Modern view, with the terminology modified according to new knowledge suggesting a pallial origin for most of the telencephalon. From (Jarvis et al. 2013).

Among other structures, a great similarity between the avian "associative" pallium (nidopallium and mesopallium) and the mammalian cortex has been recognized (Wang et al. 2010; Jarvis et al. 2013; Pfenning et al. 2014; Sayol et al. 2016). Thus, it is now possible to relate avian brain structures with acquired knowledge in mammals at the molecular level to investigate their roles in cognition.

Apart from studies on songbirds, few behavioral studies conducted on wild animals have involved neurobiological analysis at a molecular level. Nevertheless, interesting investigations have shown the involvement of some neuropeptides in behavior including aggression, social gregariousness and attachment in mammals and birds (Hammock 2005; Goodson et al. 2006; Phelps 2010). However, these paradigms focus more on aspects of the neuroendocrinology of cognition rather than its neurobiology. The classic neuroecological case of caching chickadees also provides an excellent example of successful discoveries of neural correlates of an adaptive behavior (Sherry 2011). This research program has recently led to in-depth neuroscience investigations, showing among others that food-caching chickadees that live in harsh environments rely more on stored food, perform better in spatial memory tasks and have

larger hippocampus that is characterized by higher neurogenesis levels than those that live in more benign environments (Roth et al. 2010; Roth et al. 2012). However, apart from vocal learning and the latter studies comparing populations, no research has been done at the species level on the fine-level neurobiology of cognitive abilities like innovation.

Finally, it has been shown that glutamate receptors are highly conserved in vertebrates (McEntee and Crook 1993; Myhrer 2003; Wada et al. 2004; Marek 2010; Herold et al. 2011). In birds, the individual role of each receptor has yet to be demonstrated, but non-specific blockade of NMDA receptors in pigeons reduces performance on several tasks (Herold 2010; Lengersdorf et al. 2015a). Together, this evidence suggest that NMDA receptors are implicated in cognition in birds. We thus have good reasons to believe that they could be implicated in innovation.

Our model

The four main chapters of this thesis involve the Barbados bullfinch *Loxigilla barbadensis*. It is an extremely bold and opportunistic species that belongs to the family *Thraupidae*, which shows a high level of innovation (Appendix III). In rural areas, it mainly feeds on seeds and nectar (Evans 2009). In urbanized areas, it takes advantage of feeding opportunities brought by humans, such as food on restaurant terraces, leftovers in parks and trash bins (**Figure 3**; Appendix III). On restaurant terraces, it was seen removing lids off sugar jars and opening sugar packets (Reader et al. 2002), an innovation that will be re-examined in Chapter 1.

Its presence throughout the island of Barbados, both in rural and urban areas, together with its innovative nature, makes *L. barbadensis* an ideal species to study the effect of urbanization on innovative problem-solving skills (Chapter 2). In accordance with its innovative aptitude in the wild, *L. barbadensis* performs extremely well in problem-solving tasks in captivity (Chapter 2 and Chapter 3). In the first three chapters of this thesis, I will investigate how innovation appears in *L. barbadensis*, which

captivity tasks are accurate correlates of innovativeness and how innovativeness relates to urbanization in this species.

To investigate the neural correlates of innovation, I will compare *L. barbadensis* with its sister species *Tiaris bicolor* (Chapter 4). Although both species are closely related, sympatric and similarly territorial, they display very divergent foraging strategies: unlike *L. barbadensis*, *T. bicolor* is shy and extremely conservative (**Figure 3**).

Figure 3. Differences in *L. barbadensis* and *T. bicolor* foraging strategies. *L. barbadensis* and *T. bicolor* overlap in their foraging in the field, but *L. barbadensis* (bottom) also show opportunistic and bold interest in anthropogenic foods in the same habitat where *T. bicolor* (top; male left, female right) feed only on grass seed. The two photos were taken from the exact same spot by simply pivoting the camera 90 degrees.

Considering their many similarities but also their extreme divergence in innovativeness, the two species are the ideal pair to study the divergence of innovation at the neural level. I acknowledge that the use of only two species can limit the interpretation of an eventual association between a neural property and a behavior. However, to date, very little is known on the neural bases of problem-solving. As a

starting point, comparing a pair of species that profoundly diverge in that particular trait (but that are similar for other traits) is arguably the best approach and it should lead to groundbreaking discoveries, which can later be confirmed in other species.

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Connecting statement

This first chapter presents an example of a feeding innovation used by Barbados bullfinches in the field. The innovation, taking sugar packets from restaurant tables, was first described in 2002. Here, we examine the persistence over time of the innovation in the place where it was first seen and map its spatial distribution to determine if it is continuous or not, which would suggest cultural transmission or independent invention respectively.

Chapter 1.

Independent appearance of an innovative feeding behaviour in Antillean bullfinches.

Ducatez S*, Audet J.-N.* and Lefebvre L

*Both authors contributed equally to the study

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Abstract

Behavioural innovations have been largely documented in birds, and are thought to provide advantages in changing environments. However, the mechanisms by which behavioural innovations spread remain poorly known. Two major mechanisms are supposed to play a fundamental role: innovation diffusion by social learning, and independent appearance of the same innovation in different individuals. Direct evidence for the independent emergence of the same innovation in different individuals is however lacking. Here, we show that a highly localized behavioural innovation previously observed in 2000 in Barbados, the opening of sugar packets by *Loxigilla barbadensis* bullfinches, persisted more than a decade later and had spread to a limited area around the initial site. More importantly, we found that the same innovation appeared independently in other, more distant, locations. On the island of St-Lucia, 145 km from Barbados, we also found that the sister species of the Barbados bullfinch, the Lesser-Antillean bullfinch *L. noctis* developed the same innovation independently. Finally, we found that a third species, the Bananaquit *Coereba flaveola*, exploited the bullfinches' technical innovation to benefit from this new food source. Overall, our observations provide the first direct evidence of the independent emergence of the same behavioural innovation in different individuals of the same species, but also in different species subjected to similar anthropogenic food availability.

Keywords: behavioural innovation, cognition, behavioural flexibility, social learning, Barbados

Introduction

Innovations are novel behaviours that represent new solutions to ecological problems (Kummer and Goodall 1985), so that individuals/species exhibiting a higher innovation propensity are expected to be more likely to cope with new environmental conditions (Sol et al. 2005a). In this context, innovations are expected to be driven by environmental changes (Reader 2007; Ramsey et al. 2007) and many reported innovations are indeed responses to human-induced environmental changes (Lefebvre et al. 1997; Lefebvre et al. 2001; Reader and Laland 2002).

Despite the potential importance of innovations for conservation (McDougall et al. 2006) and evolutionary (Nicolakakis et al. 2003; Sol et al. 2005b) issues, the mechanisms leading to the spread of behavioural innovations remain poorly understood. Three mechanisms have been proposed: (i) independent appearance of the same innovation in different individuals; (ii) social learning, i.e. the diffusion of innovations through direct observation of innovative individuals by non-innovative ones; (iii) natural shaping, when the action of an innovator on the environment subsequently favours individual learning by another individual without any direct contact between the innovator and the second individual (Galef 1992). Experiments on black-capped chickadees (Sherry and Galef 1984) and titmice (Kothbauer-Hellmann 1990) suggest that all three may have contributed to the classical case of milk bottle opening by Paridae in Britain and Ireland (Fisher and Hinde 1949; Hinde and Fisher 1951, 1972), but do not bring direct evidence of the independent emergence of behavioural innovations.

Distinguishing the relative importance of the three mechanisms can be challenging because of the difficulties of documenting the independent appearance of one particular innovation in the field. Current evidence for the independent origin of innovation is based on observations of the simultaneous emergence of the same innovation in distant places, but these observations did not rule out the possibility that it resulted from dispersal of innovators. Thomson et al (1996) have suggested that the appearance of a foraging innovation (nectar robbing) in territorial blue tits *Parus caeruleus* in two separate areas in Oxford was the result of different individuals independently adopting the same behaviour, but the hypothesis that innovators dispersed

and transmitted the innovation from one area to the other could not be eliminated. Further evidence for multiple independent origins of an innovation was also provided, albeit indirectly, by Lefebvre's (Lefebvre 1995) re-analysis of Fisher and Hinde's (1949) data. The distance-by-time function for all areas where bottle opening was noticed suggested independent innovation by many birds rather than a cultural wave of advance from the site and date where the behaviour presumably originated. Still, those conclusions are based on indirectly inferred data, and no direct evidence for an independent innovation by different birds was brought in this case.

Direct evidence for the independent emergence of the same innovation in different areas is still lacking. Here, we follow up on a previously reported foraging innovation, the opening of sugar packets by Barbados bullfinches (now *Loxigilla barbadensis*, previously *Loxigilla noctis*) at a single site in Barbados (Reader et al. 2002). This innovation requires relatively complex motor skills, and only bullfinches were observed performing this task in Barbados, despite the presence of other opportunist species such as the Carib grackle *Quiscalus lugubris* on the island. Barbados bullfinches are considered as rather territorial (Reader et al. 2002), although territory size and movements in this species are poorly known, and their life expectancy is estimated at 4 years (www.birdlife.org). We first investigated whether, more than a decade later, the behaviour still existed at the site where it was first seen, and if it had spread around the initial site. We then enlarged the study zone to identify new areas where the same innovation could be potentially present. We took advantage of the observation of bullfinches opening sugar packets of a different colour at a new location (where this behaviour had not been previously recorded) to test whether these birds were interested in the sugar packets found at the initial location. If not, it would strongly suggest that the behaviour independently appeared at the two sites. Lesser Antillean bullfinches *Loxigilla noctis* living in St. Lucia, an island situated 145 km north-west of Barbados, were also opportunistically observed. This species is closely related to the Barbados bullfinch, the speciation dating from only ~ 0.2 – 0.7 m.y. ago (Buckley and Buckley 2004). Finally, we report exploitation by bananaquits *Coereba flaveola* of sugar packets previously opened by bullfinches, a case of interspecific scrounging.

Methods

Sites were examined between February 25 and April 30, 2012, which coincides with the main tourist season, and thus with the peak of food availability for the very tame and opportunistic Barbados bullfinch around terraces and restaurants. Eleven sites were selected in the vicinity of the Colony Club (see Figure 1), where the sugar packet opening behaviour was initially noted in 2000. We focused on surrounding restaurant terraces, but also included one picnic area south of the Colony Club and two sites without anthropogenic food sources north of the Colony Club in order to pinpoint the area where the innovation might have spread. We also examined the nearest area north of the Colony Club where anthropogenic sources of sugar might be available (Royal Pavilion), which was ca. 1 km away (see Figure 1). Each site was prospected once in the morning (between 8 and 10 a.m.) and once in the afternoon (between 4:30 and 6:30 p.m.) on different days. At each site, we placed six sugar packets within a radius of 5 m and observed from a distance of at least 2 m. We obtained sugar packets similar in colour (white) and design (6 cm by 4 cm) to those used at the Colony Club. Each observation lasted a maximum of 1 h when no sugar packet opening behaviour was observed. We also included one site situated more than 500 m from any restaurant terrace in order to test whether individuals less familiar with anthropogenic food sources would open sugar packets. At all sites, bullfinches came within 10 cm of at least one packet. We were not able to identify the sex of the birds as Barbados bullfinches are monomorphic (Buckley and Buckley 2004). Finally, we observed Lesser Antillean Bullfinches on the island of St. Lucia (145 km from Barbados) in the morning of April 24, 2012.

Results

Sugar packets were opened at three different sites in the immediate vicinity of the initial place where the behaviour was first recorded in 2000 (Figure 2A). Bullfinches opened at the Colony Club terrace, the Heron Bay gap (north of the Colony Club), and the Coral Reef terrace (south of the Colony Club) (Figure 1). On a fourth site, the Chattel Village, 1200 m from the Colony Club, the birds did not attempt to open the white sugar packets we offered, but we observed them opening brown-coloured ones available on their tables. On the 5 sites sampled situated between the Chattel Village and the Colony Club,

bullfinches did not attempt to open sugar packets during our observations. The Chattel Village and the Colony Club were the two only places in the sampled sites where sugar packets were commonly available outside of our experiments. Although the bullfinches were not identified with legbands, we observed two to three birds opening sugar packets at the same time at the Coral Reef terrace, the Heron Bay gap and the Chattel Village

At all sites where the birds opened the packets, they did it within the first five minutes of observation, and the method used by the birds to succeed was very similar. Individuals first examined the packet and flipped it over, as if they were observing whether the packet was already open or not, and then either flew away carrying the packet in their beak or started immediately to peck at it, eventually piercing it and eating the sugar inside (see video 1 in the Supplementary Material). At two of the sites where bullfinches opened sugar packets (Coral Reef Club terrace and Heron Bay gap), we also observed bananaquits (*Coereba flaveola*) feeding from sugar packets already opened by bullfinches, as previously observed by Reader et al. (2002). Bananaquits did not manipulate sugar packets at the other sites where they were offered, although they were observed within 4 m of the packets at two places, the Surfside and the Royal Pavilion restaurant terraces, where they fed on other anthropogenic food sources. Bananaquits might be technically unable to pierce packets because of their long, thin curved beak adapted to feeding from flowers. Finally, we observed two different male Lesser Antillean Bullfinches (*Loxigilla noctis* is sexually dimorphic) opening sugar packets at Anse Chastanet (hotel terrace) in St. Lucia (see Figures 1 and 2B and video 2 in Supplementary Material).

Discussion

The sugar packet opening behaviour observed in 2000, restricted only to the Colony Club (Reader et al. 2002), was still observed in 2012. Surprisingly, however, the behaviour has spread little (less than 200 m) from the initial site, despite that the area is full of restaurants and hotels. As sugar packets are not normally distributed at the Coral Reef or in the Heron Bay gap, it is likely that the birds opening the packets at these locations developed this behaviour at the Colony Club terrace. Although we were not able to identify the different birds, two individuals recognizable by plumage features

and avian pox lesions were observed at the Coral Reef terrace but never on the other sites, suggesting that the movements were limited between these sites, and that different birds were observed at the three sites around the Colony Club. We could expect individuals from territories near to the Colony Club terrace to sometimes visit the terrace, or to have occupied this territory in the past, acquiring the capacity to open sugar packets. These birds may have independently developed the innovation, or learned socially from their conspecifics. Even if the type of dispersal we observe (see Figure 1) favors the hypothesis of social learning around the Colony Club, it remains impossible to determine whether the innovation spread through social or asocial mechanisms at these 3 sites. As proposed by Reader et al. (Reader et al. 2002), the territoriality of the birds may have restricted the spread of the novel behaviour to a larger area.

We also found that bullfinches were able to open sugar packets of a different colour at the Chattel Village, at a distance of 1000 m from the Coral Reef, the nearest place where bullfinches were observed opening sugar packets around the Colony Club. As the sugar packet opening behaviour was not observed between these two sites, the behaviour either arose independently at the two sites or was brought by an immigrant from one site to the other. However, the fact that the Barbados bullfinches from the Chattel Village did not attend to the white packets we offered, similar in colour to those routinely available at the Colony Club, but only to the brown ones available at that site, suggests that the behaviour appeared independently at the two places. We were also informed of a bullfinch opening a white sugar packet at Accra Beach in April 2011 (Dr. R. Russel, pers. comm.), 13-14 km south of the areas we canvassed here (see Figure 1), suggesting the existence of a third independent appearance of this behaviour. We can however not rule out the hypothesis that the opening of sugar packets on this third site resulted from the dispersal of an innovator. Finally, the observation of two Lesser Antillean bullfinches opening sugar packets in St. Lucia clearly demonstrates the independent appearance of the same innovation in two species.

The interest of bananaquits in sugar packets specifically at places where bullfinches are observed opening them suggests the existence of an association between both species, where bananaquits scrounge the innovative behaviour of bullfinches to

obtain otherwise inaccessible food (Giraldeau and Caraco 2000). Indeed, bananaquits were not observed opening sugar packets, probably because their beak morphology makes them technically unable to do so. Scrounging of an innovative behaviour by heterospecifics suggests that even species that are technically unable to perform an innovation could benefit from the behaviour of other species to enlarge their own foraging repertoire.

This is the first study that clearly demonstrates the independent appearance of the same innovation in different individuals within a species and in two different species. Our observation thus tends to confirm the expectation that independent appearance of innovation may be of strong importance in innovation spread. It remains however difficult to evaluate the relative importance of the different mechanisms responsible for innovation spread, both within the considered species and in other species. It is likely that both the species ecology (such as territoriality) and the distribution of an innovation source (like in our example the sugar packet distribution) will largely affect how social and asocial mechanisms will drive the spread of an innovation, so that spread mechanisms may largely vary according to species, populations and innovations. Nevertheless, our findings indicate that independent appearance of an innovation exists, and future observations should carefully address that possibility when analyzing innovation mechanisms.

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Figures

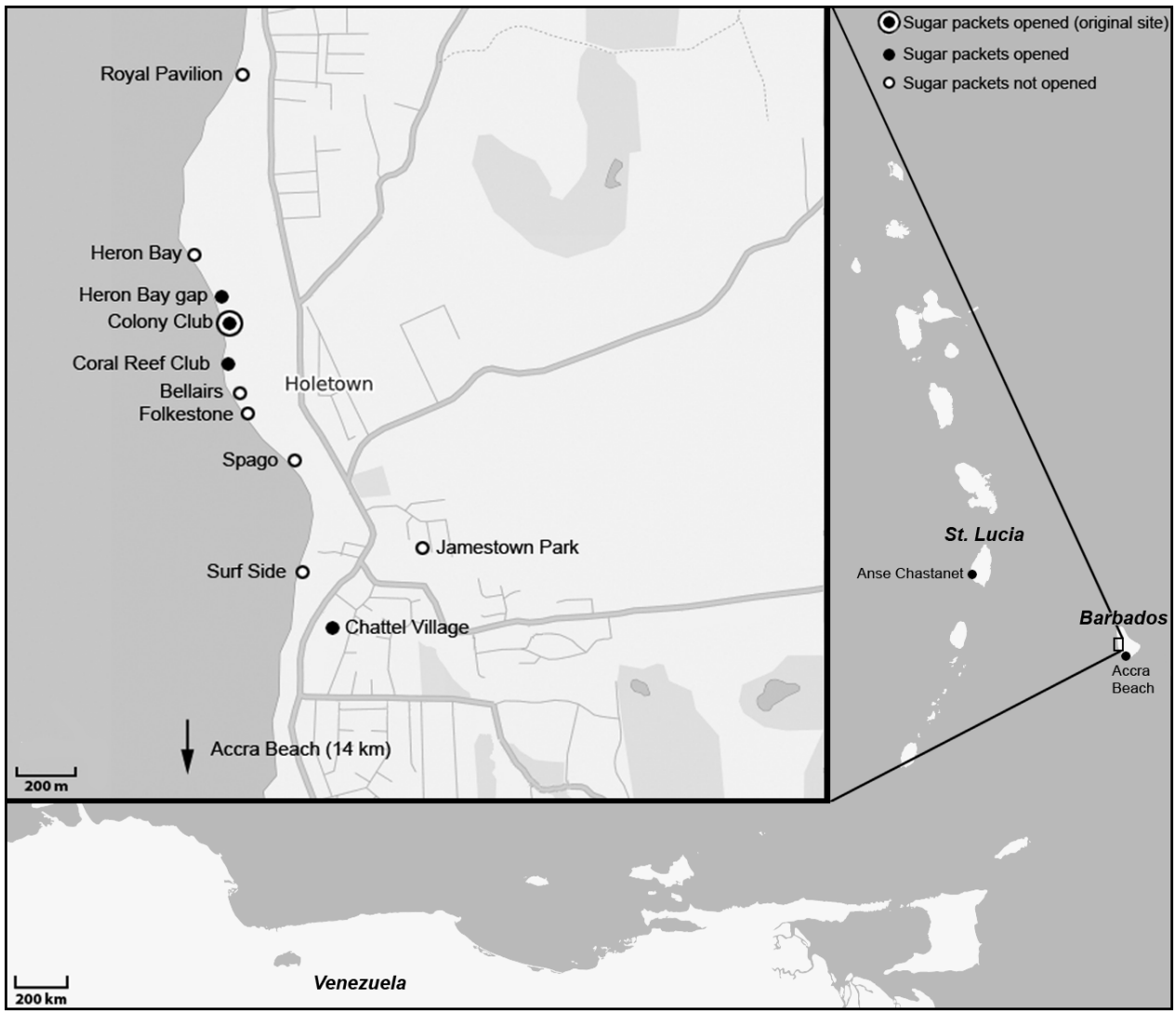

Figure 1. Sites prospected for sugar packet opening behaviour in Antillean bullfinches *Loxigilla barbadensis* and *Loxigilla noctis*.

A

B

Figure 2. Sugar packet opening behavior. A) Barbados bullfinch Loxigilla barbadensis opening a sugar packet at the Coral Reef terrace, Barbados. B) Male lesser Antillean bullfinch Loxigilla noctis opening a sugar packet at Anse Chastanet, St. Lucia.

Connecting statement

In chapter 1, we aimed at characterizing how innovations appear and how they spread. We found that they can indeed persist over time and spread to some extent, but the same innovation can also appear multiple times independently. All the sites where we observed the innovation were in urban areas. The innovation was in fact a direct consequence of an opportunity offered by humans (presence of sugar packets on terraces).

In chapter 2, we test the hypothesis that birds are more innovative in urban areas, as the frequency of innovation opportunities is higher there, and thus being innovative should be more useful in cities than in rural areas. To that aim, we used the same species that showed urban innovations in chapter 1, *L. barbadensis*. If urbanization favors innovativeness, we should observe better problem-solving skills in urban compared to rural *L. barbadensis*. We also assess immunocompetence in the same birds, as a potential trade-off with the costly investment that cognitive abilities can represent.

Chapter 2.

The Town Bird and the Country Bird: problem-solving and immunocompetence vary with urbanization.

Audet J.-N., Ducatez S. and Lefebvre L.

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Abstract

Thriving in urban habitats presumably requires changes in cognitive, behavioral and physiological traits enabling individuals to exploit new resources. It is predicted that boldness, reduced neophobia and enhanced problem-solving and learning skills might characterize urban birds compared to their rural conspecifics, while exposure to novel pathogens might require an enhanced immunity. To test these predictions, we assessed problem-solving, color discrimination learning, boldness, neophobia and immunocompetence in the bullfinch *Loxigilla barbadensis*, a highly opportunistic and innovative endemic bird in Barbados, wild-caught from a range of differently urbanized sites. Birds from urbanized areas were better at problem-solving than their rural counterparts, but did not differ in color discrimination learning. They were also bolder but, surprisingly, more neophobic than rural birds. Urban birds also had an enhanced immunocompetence, measured with the PHA antigen. Our study sheds light on the trade-offs acting on animals exposed to changing environments, particularly in the context of urbanization.

Introduction

Urbanization is considered one of the most severe threats for biodiversity and has been shown to dramatically alter animal abundance and diversity through the extinction of native species or changes in their distribution (Case 1996; Crooks 2004; Sol et al. 2014). Characterizing traits that enable successful species to thrive in urban habitats is a key to a better understanding of the evolution of urban ecosystems. Species that are abundant in urban areas are expected to be characterized by behavioral and physiological traits that increase their ability to survive and reproduce in such environments (McKinney and Lockwood 1999; Evans et al. 2009). Boldness and “behavioral flexibility” are, according to a review by Lowry et al. (2013), the two most important factors that affect a species' response to urban environments. Boldness is mostly measured by how well animals tolerate proximity to humans, however it is not clear what Lowry et al. (2013) meant by “behavioral flexibility”, as they included several seemingly unrelated behaviors in their definition (e.g. problem-solving and song pitch level). In any case, there is overwhelming evidence on dozens of avian species that urban populations tolerate a

closer approach by humans than do rural populations of the same species (Cooke 1980; Knight et al. 1987; Valcarcel and Fernández-Juricic 2009; Evans et al. 2010; Lowry et al. 2011; Atwell et al. 2012; Møller and Tryjanowski 2014). However, but results on problem-solving and tolerance of novel environmental features are both much rarer and less clear. Problem-solving differences between conspecifics from urban and rural populations are sometimes weak (e.g. urban house sparrows better on only one of four tasks, Papp et al. 2014) or confounded by other variables (e.g. body size, Papp et al. 2014), while urban birds are often more or equally neophobic towards novel environmental features compared to rural ones (Echeverría et al. 2006; Echeverría and Vassallo 2008; Bókony et al. 2012; Miranda et al. 2013).

In this paper, we examine differences between urban and rural Barbados bullfinches (*Loxigilla barbadensis*), an endemic opportunistic and innovative species that is very successful in urban areas, but which is also abundant in less disturbed areas of Barbados (Webster and Lefebvre 2000; Reader et al. 2002; Ducatez et al. 2013). The island of Barbados shows a strong gradient of human disturbance, thus providing an excellent environment to study the effects of urbanization. In addition to boldness, neophobia and innovative problem-solving, we test two variables that could be linked with urbanization, enhanced immunocompetence and faster reversal learning. Reversal learning is the classical measure of "behavioral flexibility" in psychology. Contrary to obstacle removal tasks in innovative problem-solving, much is known about the neural (Lissek et al. 2002), genetic (Krugel et al. 2009) and psychological (Cools et al. 2002) mechanisms of reversal learning, an advantage that could ground ecological studies of flexibility in a wider literature. Tebbich and Teschke (2014) have used reversal learning to show that Darwin's finches from an arid zone that goes through sharp variation in dryness make fewer reversal errors than conspecifics from a less variable cloud forest. As far as immunocompetence is concerned, comparative studies on both birds (Garamszegi et al. 2007; Soler et al. 2011; Vas et al. 2011) and primates (McCabe et al. 2015) have shown that increased contact with pathogens is one of the costs of innovative foraging. Enhanced immunocompetence might thus be one of the responses that behaviorally flexible animals develop or evolve given the wide and novel array of

pathogens they encounter as a result of invasive (Sol, Duncan, et al. 2005) and generalist (Ducatez et al. 2015) lifestyles that go with flexibility.

Methods

Subjects. Fifty-three Barbados bullfinches were captured in mist nets between February and May at eight different sites throughout the island of Barbados that were selected in order to obtain a wide range of urbanization rates (Table 1). Urbanization rates were calculated using the percentage of anthropogenic structures in a 1 km^2 area around the point of capture (as in Jacquin et al. 2013, Table 1, also see map: Figure S1).

Morphological measurements. Morphological measurements were taken at capture on all 53 birds by the same person (JNA); measurements were taken three times in succession on each bird and the mean value of the three measures was used in the analyses below. Individuals were weighed using a digital pocket scale (precision to 0.1 g). We measured tail length as the length of the longest straightened rectrix using a metal ruler (precision to 0.5 mm). Wing length was taken with a raised-end ruler as the length of the unflattened wing chord (precision to 0.5 mm). Calipers were used to measure the metatarsi, bill, and head (precision to 0.05 mm). Metatarsal length was measured from the intertarsal joint to the last scale before the toes. Bill length was measured from the tip to the anterior edge of the nostril. Head length was measured from the anterior edge of the nostril to the back of the head following the angle of the bill (Audet et al. 2014). Residuals of body weight against wing length were used as a proxy of body condition.

Captivity conditions. Birds were housed in individual cages (H: 92 cm x W: 73.5 cm x L: 81 cm) that were visually but not acoustically isolated from each other in an indoor aviary. Tests started after a 2-day habituation period during which the birds were fed *ad libitum*. On the day of the first behavioral test, birds were food-deprived overnight for 14 h. Behavioral tests began at 9:00, a 1-hour pause was given at noon during which they were given 10 min to feed and the tests stopped at 16:00. Birds were then fed *ad libitum* until the next overnight deprivation (starting at 19:00). Birds were given a commercial mix of finch seeds when fed before and between the tests and also as a reward for behavioral tests. During the tests, the observer (JNA) was hidden behind an opaque curtain and observed through small holes at a distance of 5 m from the cages.

Birds lost on average 0.7 g of their body weight at capture, which represents a mean of 4.3 % of their initial weight. At the end of the captivity period (7 days), birds were released at their initial site of capture. Three out of the 53 birds died from unknown causes during captivity testing (one female from White Hill, one female from Swans and one male from Bellairs); they were excluded from analyses. All experiments were conducted according to Animal Use Protocol 2013-7140, approved by the McGill University Animal Care Committee and permit 8434/56 from the Natural Heritage Department of the Barbados Ministry of Environment and Drainage.

Behavioral tests. Tests were always given in the same order to reduce the potential biases emerging from habituation (see Ducatez et al. 2015 for a detailed explanation). Behavioral tests started on day 3 of captivity with boldness assessment. Birds were presented with an open Petri dish full of seeds (same dish and food as during the habituation period) and the experimenter hid behind the curtain until the bird had fed (all birds fed within12 min). The same procedure was repeated on the three following days of captivity to assess repeatability of the boldness measure. On day 3, after the boldness test, neophobia was assessed by presenting a novel object beside the Petri dish until the bird fed or reached the maximum 20 min limit of the trial, in which case this 20-min latency was recorded for the individual. Neophobia was measured as the latency to feed, minus the mean latency of pre- (previous boldness measurement) and post- (additional boldness measurement) controls. Failure to remove boldness could result in a confounding of an animal's response to the novel object and to the human presenting it (Greenberg 1983). The first novel object was a 30 cm yellow stake (Figure S2A). To estimate repeatability of neophobia, we took another measure of neophobia on day 6, after changing the novel object to two brightly colored and textured balls (dog toys, 50 mm diameter) placed directly on each side of the dish (Figure S2B). As our measure of neophobia, we used the mean of the neophobia latencies obtained on days 3 and 6. There was no significant difference between neophobia measured upon the first presentation of the two objects (day 3 and 6: $p = 0.235$).

On day 3, after the neophobia trial, we assessed problem-solving ability using the lid-drawer task (see SI movie 1). A 2 cm x 3 cm x 3 cm drawer made of white plastic was

constructed with a circular opening (1.5 cm diameter) at the top covered with a lid to which a hook was attached (Figure S2C). The birds had the opportunity to gain access to food by opening the lid or by pulling the drawer. Birds were given a maximum of 15 trials each lasting 5 min. The problem-solving score was defined as the latency to succeed, which was started when the individual touched the apparatus for the first time, thus removing initial boldness or neophobia effects from the problem-solving score. The other problem-solving task, the tunnel task, was given on day 4 of captivity. It consisted of a transparent rectangular box (H: 3 cm x W: 3 cm x L: 10 cm) opened on only one side (Figure S2E: as presented to the birds and F: opened, see also Movie S2). A transparent cylindrical tube containing seeds and topped with a loose fitting white lid was inserted at the closed end of the tunnel and a wooden stick was attached to it so that the birds had to pull on the stick to get the tube out of the tunnel. Once the tube was out, the bird had to remove its lid to gain access to seeds. Birds were given a maximum of 15 trials each lasting 5 min and problem-solving latency was measured in the same way it was for the lid-drawer task.

On day 5 of captivity, a color discrimination task was made to first assess acquisition learning ability. The test apparatus consisted of two Petri dishes (same as the one used for the boldness assessment), each inserted in a wooden platform (10 x 10 x 10 cm) painted either green or yellow and open on one side, placed at each extremity of the cage (Figure S2D). A "color bias" trial was first made, where the bird was allowed to eat from one dish, and the color of the wooden platform chosen by the bird was considered as its preferred color. The other color thus became the rewarded one in order to control for initial color bias. The Petri dish inside the unrewarded color contained seeds glued to the bottom of the dish, so that no difference could be seen from a distance but the seeds were impossible to remove for the birds. This task was designed to measure discrimination learning without a problem-solving or motor skill component, since the bird only had to choose a color without performing a novel motor task. Novelty was also reduced since the birds were already habituated to feed from similar (but not color associated) Petri dishes. On each trial, the two platforms were introduced simultaneously inside the cage. The bird was given up to 5 min to choose a dish. If the bird chose the rewarded color, it was allowed to feed for 15 sec. If the bird chose the unrewarded color,

the two platforms were immediately removed by the experimenter. A "choice" is defined as the first peck movement towards the seeds in the Petri dish on either side. Since the seeds were glued on the unrewarded side, the peck yielded no reward on this side. The location of the rewarded platform was switched at each trial to control for spatial preference. The success criterion was reached once the birds chose successively the correct (rewarded) color for seven consecutive trials (Boogert et al. 2010). On the day after this criterion was reached, we assessed reversal learning. We switched the rewarded color and tested the birds in the same way we did in the acquisition phase. On the first reversal learning trial, all birds initially chose the previously rewarded color (which was incorrect at this stage), indicating that they effectively learned the color stimuli, and not a potential perceptible difference in the Petri dishes. See Figure S2 for pictures of all tasks. Upon completion of all tasks (including boldness and neophobia assessment), birds were allowed to feed for two minutes. A new task was started only when every bird had completed the previous one (either success or maximum number of trials reached). This allowed for a relatively constant food intake while keeping the birds hungry enough to be motivated.

Immunocompetence assessment. Immunocompetence was assessed using a phytohemagglutinin (PHA) injection, a measure of the cellular immune response. Measurement of PHA-induced swelling in birds is a well-established immunoecological technique that has the advantage of assessing general innate immunity (and to a lesser extent adaptive immunity) and it is easily performed in the field (Martin et al. 2006). It was performed on the last day of captivity (day 7) by subcutaneously injecting phytohemagglutinin (PHA) at a concentration of 5 μg/g (e.g. 0.033 mL of a 3 mg/mL PHA solution for a 20 g bird) in the proximal portion of the wing, as described in Martin et al. (2006). We measured swelling of the tissue with a micrometer caliper (Mitutoyo, USA) by subtracting the wing thickness before injection from thickness of the same region 21.5 ± 0.6 hours after the injection.

Sex-typing. *L. barbadensis* is monochromatic, so molecular sexing of individuals is required. Approximately 50 μL of blood was sampled by puncturing the brachial vein.

DNA was extracted from blood and PCR sexing was performed following Audet et al. (2014).

Statistical analyses. To test for an effect of urbanization on our different variables, we separated our capture sites into 'urban' (n = 4 sites; mean urbanization score = 30% ± 12%) and 'rural' sites (n = 4 sites; mean urbanization score = 3% ± 1%; see Table S1), and used this binary variable in our linear models. Normality of the data was assessed using D'Agostino-Pearson tests. The only datasets that did not follow a Gaussian distribution were the results of the two problem-solving tasks. Therefore, we computed the p-value only for mean differences of the latter variables using a non-parametric t-test (Mann-Whitney) for the data presented in Figure 1B (note however that the computed problem-solving PC1 followed a Gaussian distribution). To test for the effect of urbanization on all other variables along with all potential confounding variables, we performed linear models and then conducted stepwise variable selection until only significant effects remained. A principal component analysis (PCA) was performed on latency to solve the lid-drawer and tunnel tasks and the first component, which explained 64% of the variance (Figure S3), was used as the general problem-solving score. For all models, tarsus length (which was found to differ between rural and urban environments), sex, body weight and body condition along with urbanization were used as explanatory variables. For neophobia, we added boldness as a potential confounding variable. For problem-solving and discrimination learning models, we also added neophobia along with boldness as potential confounding variables. Correlations between each variable and percent urbanization as a continuous rather than a binary variable were also tested.

Additionally, we tested whether associations between behavioral and immunity variables varied between urban and rural populations. To that aim, we built models with proxies of cognition, problem-solving or immunocompetence as response variables, and urbanization and proxies of behavior or immunocompetence along with their interactions as fixed effects.

Finally, we also tested all models using a mixed model (LMM) approach with the capture site as a random variable; the results of the latter models are presented in SI Appendix (Results).

Repeatability was calculated using the rpt.adj function from the rptR package (Nakagawa and Schielzeth 2010) in R 3.2.1, which allows a comparison of latencies adjusted for a given parameter. For boldness, we used the latencies measured on 4 consecutive days and added the day of measurement (habituation parameter) in the model, as latencies usually decrease as the birds habituate to this test and to human presence in general. For neophobia, we used the two measures of neophobia (day 3 and 6) and added measurement day in the model. The individual ID was used as the random variable.

JMP software (SAS Institute, Cary, NC) was used to compute all linear models, SPSS Amos software (IBM, Armonk, New York) for path analyses, rptR package in R (R Core team, Vienna, Austria) for repeatability calculations and Prism 5.01 (GraphPad software, La Jolla, CA) to draw graphs.

Results

Morphology. None of the morphological traits, including body condition, differed between rural and urban birds, except tarsus length (rural birds: 0.41 mm longer than urban birds, t = 2.22, p = 0.0307), which became non-significant after Bonferroni corrections (See Table 2). To be conservative, we nevertheless included tarsus length as an explanatory co-variable in all subsequent linear models.

Temperament. Boldness and neophobia measurements were both repeatable. The computed repeatability for boldness was 0.427 (SE = 0.066, CI = [0.300, 0.548]) and was significant ($p < 0.0001$). The repeatability for neophobia was 0.350 (SE = 0.138, CI = [0.072, 0.629]) and was also significant (p = 0.0109). Boldness was higher in birds living in urban environments than in birds living in rural environments: urban birds were faster at eating after human disturbance compared to rural birds (Figure 1A, left panel). After stepwise selection of potential confounding variables, urbanization remained significant (t = - 2.91, p = 0.0056) as well as sex (mean boldness for rural females = 143 s, rural males = 43 s, urban females = 23 s, urban males = 62 s, t = 2.16, p = 0.0366) (Table 2, see Table S1 for detailed models). However, after Bonferroni corrections, boldness was no longer significantly explained by sex and only urbanization remained as a predictor of boldness (see Table 2). In contrast, neophobia was higher in urban birds

(Figure 1A, right panel). Following stepwise selection of variables, urbanization was the only significant explanatory variable for neophobia ($t = 3.55$, $p = 0.0010$) (Table 2, see Table S2 for detailed models).

Innovative problem-solving and discrimination learning. The lid-drawer task was completed by all birds from both environments. Twenty-six percent of the rural birds and fifty percent of urban birds succeeded in completing the tunnel task. Latency to succeed at the two problem-solving tasks (lid-drawer and tunnel) varied significantly with urbanization (lid-drawer: $t_{\text{non-parametric}} = -2.18$, $p = 0.0338$; tunnel: $t_{\text{non-parametric}} = -2.39$, $p = 0.0206$; Figure 1B) and were correlated with each other ($r = 0.280$, $p = 0.042$). Urban birds performed better than rural birds on the problem-solving PC1 (Figure S3B). Following variable selection in the linear models, urbanization remained as the only significant variable explaining problem-solving performance (PC1: $t = -2.94$, $p = 0.0049$, Table 2, see Table S3 for detailed models). In contrast, discrimination learning scores did not differ between birds living in the two environments (Figure 1C). For acquisition learning, models yielded no significant effect for all tested predictors (urbanization: $t = -0.036$, $p = 0.7970$, Table 2, see Table S4 for detailed models). Similarly, reversal learning did not significantly vary with urbanization nor with any other tested predictor (urbanization: $t = 0.020$, $p = 0.9835$, Table 2, see Table S5 for detailed models).

Immunity. The injection of PHA triggered a significant swelling of the skin (mean difference = 236.5 ± 21.6 µm, $_{\text{Mann-Whitney}}$ $U = 78$, $p < 0.0001$). When comparing birds from both environments, urban birds had a 2.6-fold stronger PHA reaction than rural birds (Figure 1D). Following stepwise selection of variables in a linear model, only urbanization remained as the only significant factor explaining PHA response ($t = 5.10$, $p < 0.0001$, Table 2, see Table S6 for detailed models).

All the variables previously mentioned as significant remained so after Bonferroni corrections ($n_{\text{VARIABLES}} = 7$, $p < 0.007$) except tarsus length ($p = 0.0307$) (See Table 2). Using a mixed model approach that included capture site as a random variable also resulted in the same variables remaining significant following stepwise variable selection (all $p < 0.05$, see Tables S1-S6).

Urbanization gradient. In addition to the analyses categorizing urbanization as a binary variable, we repeated our analyses using percentage of urbanization for each of our eight capture sites, yielding a continuous gradient. We then re-ran models using the same procedure we used with the binary urbanization variable, i.e. backward selection with all potential explanatory variables. The urbanization gradient was the only significant predictor of boldness ($t = -2.12$, $p = 0.0392$, Figure S4A) and neophobia ($t = 3.39$, $p = 0.0015$, Figure S4B). Latencies to solve the two problem-solving tasks were negatively related to urbanization gradient (Lid-drawer: $t = -2.38$, $p = 0.0211$; Tunnel: $t = -2.51$ $p = 0.0151$). Furthermore, when the two tasks were combined into one principal component expressing problem-solving ability (Figure S3A), it was significantly correlated with the urbanization gradient ($t = -3.16$, $p = 0.0026$) (Figure S4C). Scores for acquisition and reversal learning remained non-significantly associated with the urbanization gradient (Acquisition: urbanization $t = -0.54$, $p = 0.5948$; Reversal: urbanization $t = -0.57$, $p = 0.5709$). Immunocompetence, as measured by skin swelling following PHA injection, varied strongly with the urbanization gradient, and this was also the only variable remaining after stepwise variable selection ($t = 7.97$, $p < 0.0001$) (Figure S4D).

Interactions between behavioral and immunocompetence variables. Linear models that included interactions between urbanization and all previously measured variables (behavioral and immunological) were tested (Table S7). No significant interaction was found in any of the models (Table S7). The only significant effect found between all combinations of variables was between acquisition learning and reversal learning when including urbanization in the model ($r^2 = 0.195/0.196$, $p = 0.002$, Table S7), individuals needing fewer trials to succeed at acquisition learning also needed less trials to succeed at reversal learning.

Path Analysis. To summarize our results and test for dependencies among variables, we constructed a path analysis. We tested every plausible path between variables and the model presented in Figure 2 is the only one in which all paths were significant ($p < 0.01$, see legend for all model statistics). This suggests that, in accordance with our linear models, there is no correlation and/or interaction among the significant behavioral

variables and immunocompetence, and that urbanization is the only common driver of variation in the traits we measured.

Discussion

Animals living in urbanized habitats are likely to be advantaged by traits that allow them to profit from human-derived food sources (Sol et al. 2011). Here, we jointly measured behavioral and immunological traits predicted to affect birds' success in urban areas. We showed that, as predicted, urban bullfinches were bolder, faster at problem-solving and had a stronger immune response than rural bullfinches (Figure 1A, B and D). Contrary to our predictions, however, urban birds were more neophobic and did not differ from rural birds in discrimination learning (Figure 1A and C). Our path analysis suggests that urbanization affects each of the traits we measured independently (Figure 2). Differences in problem-solving and immunocompetence between rural and urban birds were not explained by morphology, body condition, sex, boldness or neophobia and were significant both when urbanization was considered as a continuous gradient and as a binary variable (Figs. 1B, D and S4C, D).

Urbanization is only one of the ecological contexts in which temperament, cognitive abilities and innovativeness are expected to diverge. The expectation that boldness, low neophobia and flexibility should all co-vary with ecology has also been applied to populations that experience different degrees of environmental harshness (Roth et al. 2010; Tebbich and Teschke 2014; Kozlovsky et al. 2015). Darwin's finches from a variable arid zone are more neophilic (tendency to explore novel objects) and faster reversal learners than conspecifics from a more stable cloud forest, but they are also *more* neophobic (latency to eat in the presence of a novel object) and not better at an obstacle removal problem (Tebbich and Teschke 2014). Mountain chickadees from a harsher, higher elevation are better problem solvers than conspecifics from a milder lower elevation, but *equally* neophobic (Kozlovsky et al. 2015). Black-capped chickadees from a harsh seasonal environment (Alaska) are both better problem-solvers and *less* neophobic than conspecifics from a more benign southerly environment (Kansas; Roth et al. 2010). The relationship between flexibility and neophobia is thus inconsistent in the three studies. The same inconsistency characterizes comparative

studies of urbanization, where access to new foods in novel environments (e.g. refuse at dumps with intense truck traffic, leftovers at tables with intense pedestrian traffic) should logically favor positive co-variation between problem-solving and neophobia. Sol et al. (2011) report that urban mynas are better at solving a technical innovation problem than are suburban ones, but they do not eat a new food faster; they were also *less* neophobic and more exploratory in pecking more often at the test apparatus. The study of Bókony et al. (2012) on house sparrows shows no effect of urbanization on neophobia, while our results shows that urban birds are *more* neophobic. The contradictory data on urbanization thus support the conclusions of Griffin and Guez (2014) in their review of innovation and problem-solving: neophobia does not generally co-vary with problem-solving ability. Why this is so is puzzling and warrants a closer look at both the conceptual basis of neophobia/neophilia and at the different ways of assessing it.

Despite their inclusion under the umbrella term 'behavioral flexibility', the fact that problem-solving and reversal performance do not co-vary in our study fits with the results obtained by Tebbich and co-workers in Darwin's finches (Tebbich et al. 2010; Teschke et al. 2011; Tebbich et al. 2012; Tebbich and Teschke 2014), Griffin et al. (2013) on Indian mynas, Isden et al. (2013) on spotted bowerbirds and Ducatez et al. (2015) on Carib grackles. In most problem-solving tasks, performance is measured by the speed with which an animal removes an obstacle blocking access to food. Motor acts directed at inappropriate parts of the apparatus entail minimal costs and innovative animals routinely direct a wide diversity of movements to the apparatus at a fast rate (see Griffin and Guez 2014 and Griffin et al. 2014 for discussions on the role of motor diversity in problem-solving). In contrast, reversal errors are more costly. They entail a time-out between unsuccessful trials, with the added disturbance of human intervention in tests that are not automated. These cost differences might lead to a speed accuracy trade-off as performance at problem-solving and reversal learning tasks relies on different skills. Problem-solving requires fast, diversified pecks at many parts of the obstacle, whereas reversal learning entails accurate inhibition of response to previously rewarded stimuli. It is thus not surprising that performance on the two tasks shows either no relationship (our study, Tebbich et al. 2012, Isden et al. 2013) or a negative one

(Ducatez et al. 2015; Griffin et al. 2013). Griffin and Guez (2014) conclude that obstacle removal is a valid experimental test of feeding innovations in the wild. Whether reversal learning, in particular multiple serial reversals, is also an ecologically valid test for innovativeness is open to question. Repeated, sudden reversals of the ability of stimuli to predict rewards could be a useful test of flexibility in humans, but it might not represent an ecologically relevant situation for animals forced to opportunistically switch to a new food or new technique when their usual foraging behavior does not work. In discussing the fact that innovative, tool using woodpecker finches make more reversal errors than non-tool using small tree finches, Teschke et al. (2011) suggest that extractive foraging with tools requires perseverance, but reversal learning depends on the opposite, rapid change.

In line with predictions from the literature on both birds and primates, urban bullfinches showed both better innovative problem-solving and increased immunocompetence. Møller (2009) obtained similar results in 39 urban species compared with rural congeners or relatives, with a higher innovation rate and a larger bursa of Fabricius (a key immune organ) in urban species. By definition, intra-specific comparisons like ours include fewer confounding variables than inter-specific ones and are a more direct test of urbanization effects. The ability to mount a strong immune response is only one of the physiological adaptations urban birds have been shown to have. Suburban Florida scrub jays have lower plasma corticosterone levels than woodland jays, even when the latter are supplemented with high fat, high protein food (Schoech et al. 2004). In conditions of acute stress, urban Eurasian blackbirds show a lower level of corticosterone than rural ones (Partecke et al. 2006), as well as lower levels of oxidative stress (Costantini et al. 2014). Urban and rural blackbirds further show different SNP's for SERT genes (Mueller et al. 2013), which are associated with anxiety-related traits. Finally, urban blackbirds have lower levels of blood parasites than do rural ones (Geue and Partecke 2008).

Ecological conditions that favor differences in behavior, cognition, innovation and physiology are likely to be sensitive to time and to population isolation before they lead to evolutionary divergence. Common garden experiments on populations that are

separated by vast distances and long-term environmental differences provide the best evidence for evolved adaptive responses. This is the case for Alaskan and Kansas populations of black-capped chickadees studied by Roth, Pravosudov and co-workers (Roth et al. 2010; Roth et al. 2012; Pravosudov et al. 2013). Eurasian blackbirds have been urbanized since the 1820's and genetic differences with woodland conspecifics appear to have evolved independently in several areas of Europe (Mueller et al. 2013). Barbados is a very small island and it would be surprising if urban and rural bullfinches were geographically isolated, even if the island has a high population density and the original vegetation of the island has been destroyed and replaced by sugar cane and other anthropogenic plants for over 350 years. Enhanced boldness, problem-solving and immunocompetence in urbanized bullfinches might all be experience-driven responses to environmental variation in food, human disturbance, and pathogens. Individuals with different phenotypes might also choose habitats on the basis of traits that provide the best context-dependent benefits, in the same way that longer- and shorter-winged Zenaida doves (Sol, Elie, et al. 2005; Monceau et al. 2011) in Barbados feed at sites where territorial defence or group feeding is favored by food distribution (Goldberg et al. 2001). At temporal and spatial scales where selection is unlikely, as is probably the case for Barbados bullfinches, or at scales where long term trends and isolation might lead to genetic divergence, urbanization is one of the key situations that can help us understand how some animals respond positively to anthropogenic change.

Acknowledgements

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Figures

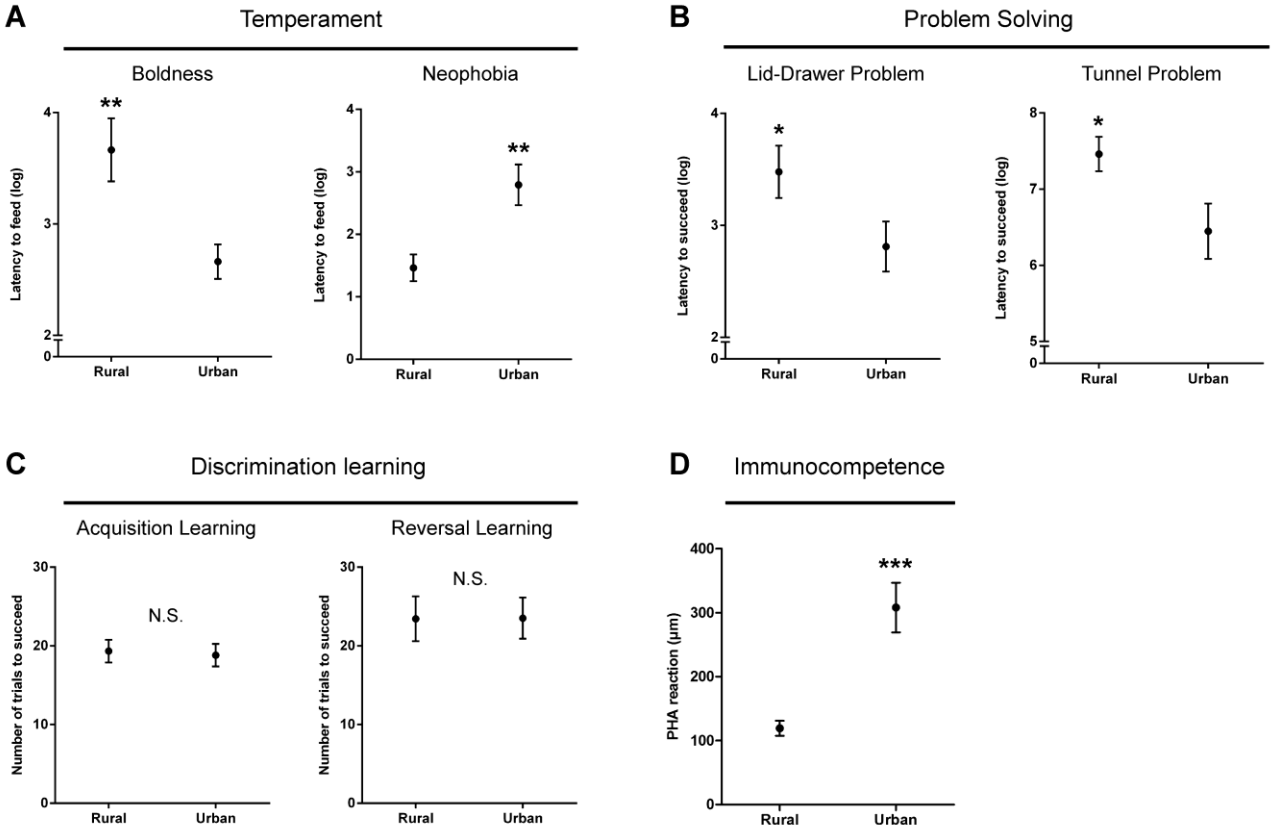

Figure 1. Behavior and immunity in urban versus rural environments. A) Temperament. Boldness (latency to feed following human disturbance) is higher in birds coming from urban environments than birds from rural environments ($p = 0.0056$). Neophobia (average of latency to feed in the presence of a two different objects on 2 different days) is higher in urban birds compared to rural birds ($p = 0.0010$). B) Problem solving. In both problem solving tasks, the latency to succeed is lower in urban individuals compared to their rural counterparts (Lid-drawer $p = 0.0338$; Tunnel $p = 0.0206$). C) Discrimination learning. The number of trials to succeed in acquisition and reversal learning does not significantly differ between rural and urban birds (Acquisition learning $p = 0.7970$; Reversal learning $p = 0.9835$). D) Immunocompetence. Intensity of reaction following PHA injection is higher in urban than in rural birds ($p < 0.0001$).

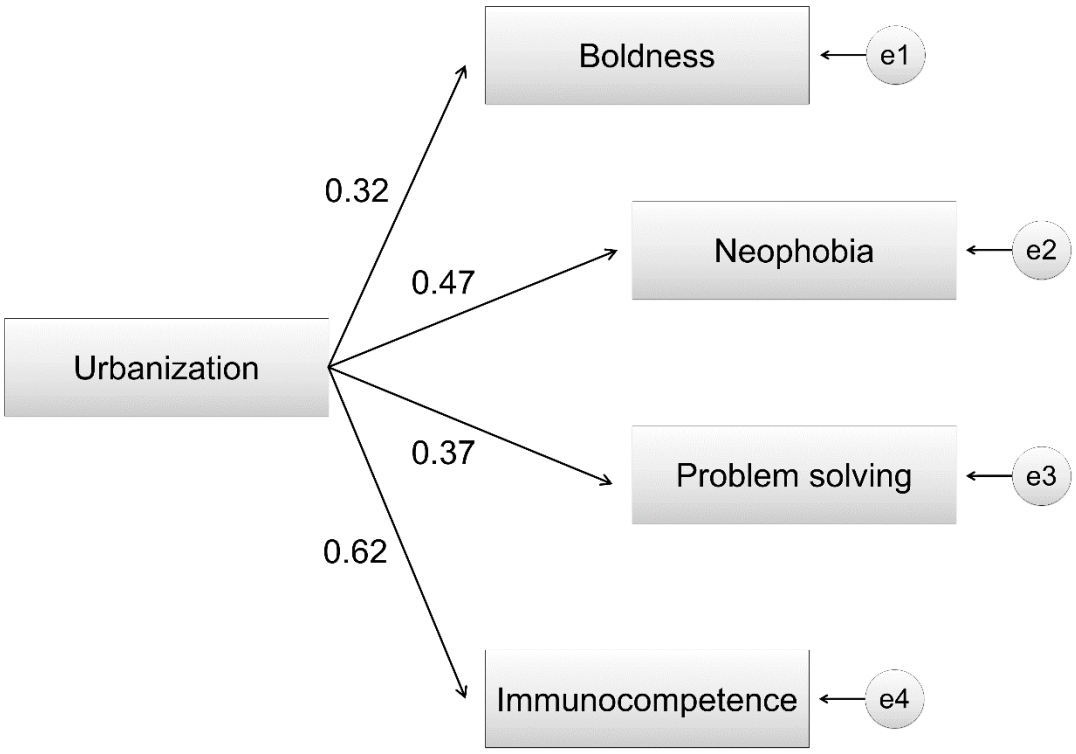

Figure 2. Path analysis. Path analysis showing dependencies between variables. Every plausible path was tested and this model is the one that maximized the number of variables, and for which all paths are significant. The model suggests that behavioral variables and immunocompetence are not directly affecting each other and that urbanization is the main variable influencing the measured variables. Variables e1 to e4 represent the error terms. Model statistics: Chi-square = 1, 302; Degrees of freedom = 6; Model probability level = 0.972; AIC = 29.302; BCC = 32.662; All individual paths $p < 0.01$.

Tables

Table 1. Summary of captured birds and their site of capture

Site name	GPS coordinates	Anthropization	*n*
Swans	+13° 14' 10.96" , -59° 35' 17.16"	2.1%	8
Bruce Vale	+13° 13' 18.98" , -59° 33' 30.74"	2.4%	6
White Hill	+13° 13' 18.24" , -59° 34' 31.68"	3.7%	7
Jah	+13° 15' 18.80" , -59° 35' 14.56"	5.6%	6
Bellairs	+13° 11' 31.21" , -59° 38' 25.20"	18.0%	6
Jamestown park	+13° 11' 18.84" , -59° 38' 7.79"	21.1%	9
Payne's Bay	+13° 9' 47.83" , -59° 38' 10.71"	25.3%	5
Bridgetown	+13° 5' 50.98" , -59° 37' 21.65"	54.7%	6
Total			**53**

$n_{'urban'} = 26$ $n_{'rural'} = 27$

Summary of all captured birds by site. Urbanization was measured as the percentage of a satellite map covered by human landmarks (roads, buildings, etc.)

Table 2. Final linear models that initially included all potential confounding variables

Final models ~ Urbanization	***n***	***r²***	***t***	***p*** *(α=0.007)*
Tarsus (length)	52	0.09	-2.22	0.0307
Boldness (latency)	52	0.15	-2.91	**0.0056** [1]
Neophobia (latency)	52	0.24	3.55	**0.0010**
Problem solving (PC1, latency)	52	0.15	-2.94	**0.0049**
Acquisition Learning (trials)	52	0.00	-0.04	0.7970
Reversal Learning (trials)	52	0.00	0.02	0.9835
Immunocompetence (thickness)	45	0.33	5.10	**< 0.0001**

Following stepwise selection of variables, only urbanization remained in all models, except when stated ([1]). Thus, t ratios (*t*) and p-values (*p*) for only urbanization effects are compiled in this table. Values in bold represent significant values following Bonferroni corrections.

[1] Sex also remained significant after variable selection ($p = 0.0366$), but not after Bonferroni correction

Supplementary information

Supplementary Figures

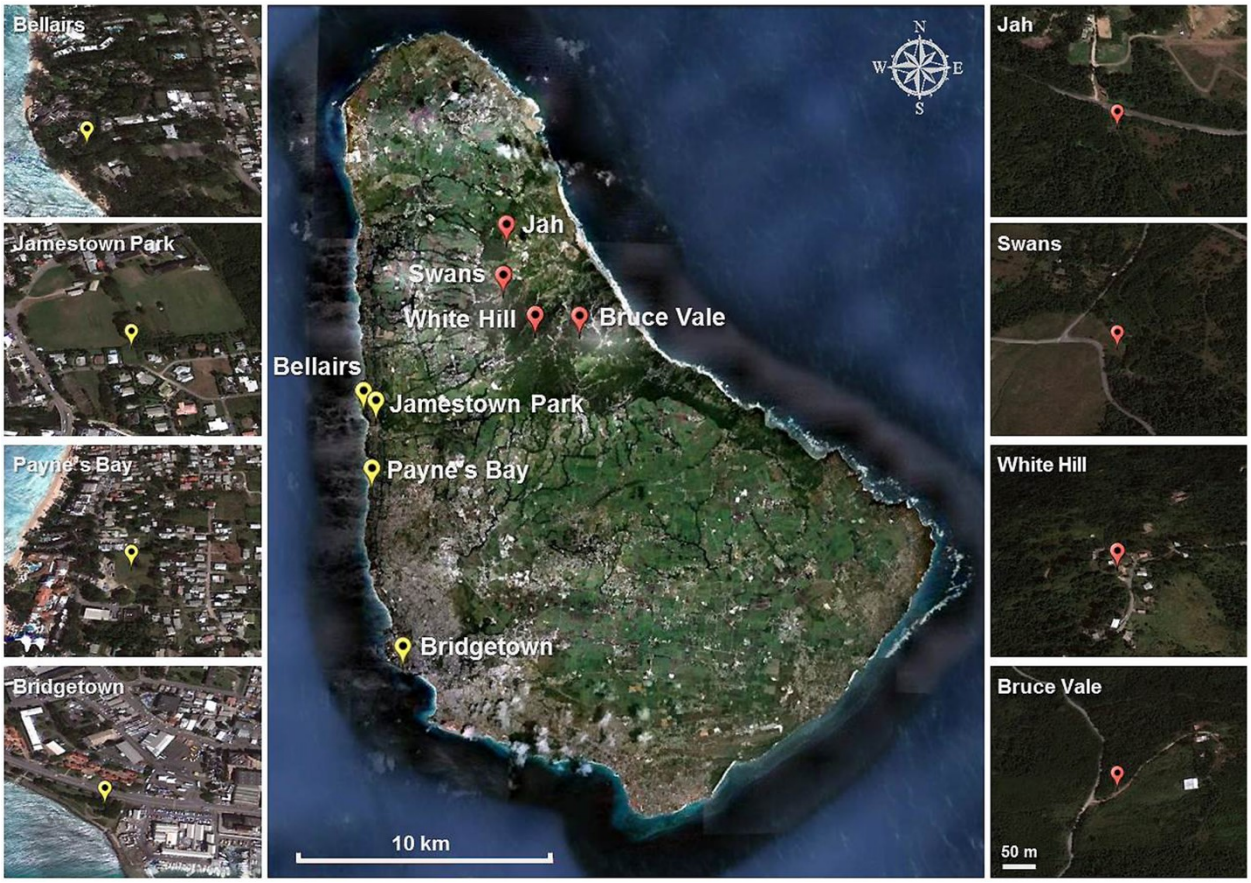

Figure S1. Map of Barbados with the 8 capture sites. Four panels on the left, yellow markers on the map: sites that are more urbanized i.e. with a percentage of anthropic features greater than 18 %. Four panels on the right, red markers on the map: sites that are more rural i.e. with a percentage of anthropic features smaller than 6 %. Source: Google Earth 7.1.2, DigitalGlobe 2014. Barbados 8/9/2013. http://www.google.com/earth [6/13/2014].

Figure S2. Behavioral tasks. A) First neophobia object along with the feeding dish. B) Second neophobia object along with the feeding dish. C) Colored platforms for acquisition learning and reversal learning. D) Lid-drawer problem solving task. E) Tunnel problem-solving task as presented to the birds and F) when it is successfully opened.

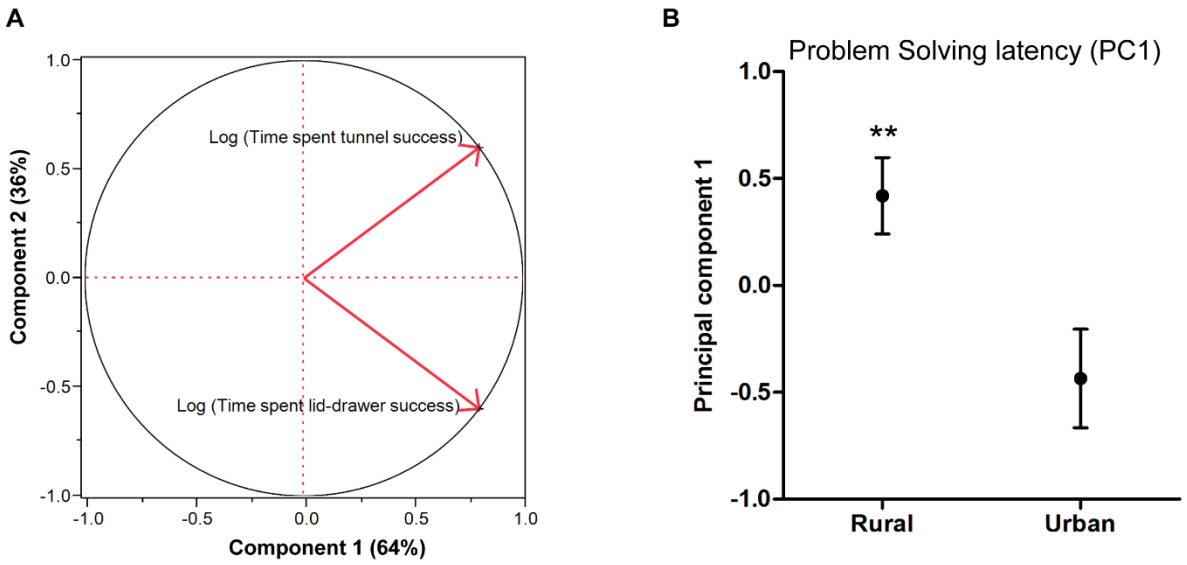

Figure S3. Problem solving tasks PCA. A) Performance on the two problem solving tasks was combined using a principal component analysis and the resulting PC1 (64% of the variance) was used for further analyses. B) Absolute PC1 was higher in urbanized birds compared to their rural counterparts ($t = 2.94$, $p = 0.0049$).

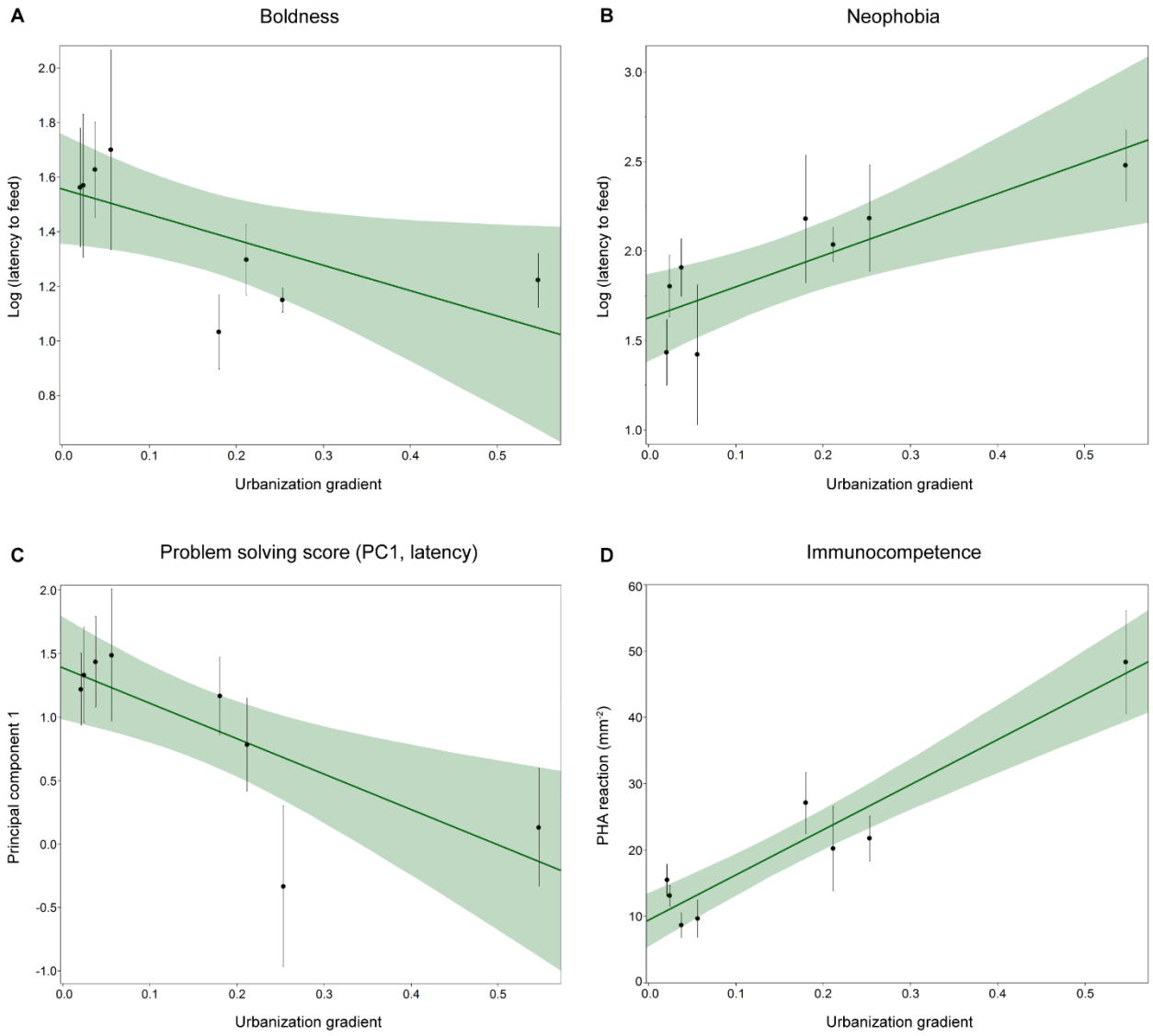

Figure S4. Behavior and immunocompetence along urbanization gradient. A) Boldness (latency to feed following human disturbance) correlates negatively with the urbanization gradient ($_{Spearman}$ r = -0.2975, p = 0.0322). B) Neophobia (latency to feed in the presence of a novel object) correlates positively with the urbanization gradient ($_{Spearman}$ r = 0.5313, p = 0.0002). C) Problem solving score (principal component 1 based on the two problem solving tasks) correlates positively with the urbanization gradient ($_{Spearman}$ r = 0.3841, p = 0.0045). D) Immunocompetence (intensity of reaction following PHA injection) correlates positively with urbanization gradient ($_{Spearman}$ r = 0.5666, p < 0.0001).

Supplementary Tables

Table S1. Models with boldness (latency) as the response variable

Linear model (LM)

Full model; adj r^2 = 0.185	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	2.041	2.372	0.86	0.3949
Urbanization (U)	-0.394	0.145	-2.72	***0.0099***
Tarsus length	-0.054	0.104	-0.52	0.6089
Sex (M)	-0.396	0.146	-2.71	***0.0101***
Body weight	0.059	0.049	1.20	0.2368
Body condition	1.164	1.884	0.62	0.5404
After variable select.; adj r^2 = 0.177				
Constant	1.692	0.116	14.59	< 0.0001
Urbanization (U)	-0.372	0.128	-2.91	***0.0056***
Sex (M)	-0.275	0.128	-2.16	***0.0366***

Linear mixed model (LMM) with site as a random factor

Full model; adj r^2 = 0.178	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	2.017	2.469	0.82	0.4190
Urbanization	-0.393	0.146	-2.69	***0.0324***
Tarsus length	-0.052	0.108	-0.48	0.6327
Sex	-0.395	0.149	-2.66	***0.0116***
Body weight	0.058	0.053	1.10	0.2823
Body condition	1.148	1.927	0.60	0.5551
After variable select.; adj r^2 = 0.072				
Constant	1.685	0.105	16.02	< 0.0001
Urbanization (U)	-0.362	0.104	-3.48	***0.0103***
Sex (M)	-0.270	0.131	-2.06	***0.0455***

Table S2. Models with neophobia (latency) as the response variable

Linear model (LM)

Full model; adj r^2 = 0.155	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	-2.443	3.169	-0.77	0.4468
Urbanization (U)	0.618	0.214	2.89	***0.0072***
Tarsus length	0.183	0.139	1.32	0.1979
Sex (M)	-0.197	0.204	-0.97	0.3412
Body weight	-0.016	0.066	-0.23	0.8159
Body condition	2.498	2.390	1.05	0.3043
Boldness	0.227	0.203	1.12	0.2732
After variable select.; adj r^2 = 0.216				
Constant	1.464	0.242	6.04	< 0.0001
Urbanization (U)	1.329	0.375	3.55	***0.0010***

Linear mixed model (LMM) with site as a random factor

Full model; adj r^2 = 0.155	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	-2.470	3.277	-0.75	0.4572
Urbanization	0.636	0.213	2.99	***0.0151***
Tarsus length	0.183	0.144	1.27	0.2139
Sex	-0.191	0.215	-0.89	0.3810
Body weight	-0.016	0.071	-0.23	0.8242
Body condition	2.287	2.496	0.92	0.3668
Boldness	0.246	0.218	1.13	0.2684
After variable select.; adj r^2 = 0.090				
Constant	1.443	5.135	7.84	0.0005
Urbanization (U)	1.365	7.984	4.52	***0.0020***

Table S3. Models with Problem solving PC1 (latency) as the response variable

Linear model (LM)

Full model; adj r^2 = 0.023	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	-9.195	9.568	-0.96	0.3440
Urbanization (U)	-0.831	0.497	-1.67	0.1045
Tarsus length	0.128	0.307	0.42	0.6789
Sex (M)	-0.707	0.460	-1.54	0.1347
Body weight	0.391	0.454	0.86	0.3964
Body condition	-13.641	18.892	-0.72	0.4757
Boldness	-0.049	0.380	-0.13	0.8988
Neophobia	0.231	0.400	0.58	0.5682
After variable select.; adj r^2 = 0.128				
Constant	0.419	0.203	2.06	0.0445
Urbanization (U)	-0.854	0.290	-2.94	***0.0049***

Linear mixed model (LMM) with site as a random factor

Full model; adj r^2 = 0.164	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	-9.25	9.5314	-0.97	0.3411
Urbanization	-0.79	0.5612	-1.40	0.2048
Tarsus length	0.15	0.3163	0.48	0.6359
Sex	-0.68	0.4799	-1.41	0.1684
Body weight	0.36	0.4704	0.76	0.4561
Body condition	-14.99	18.8760	-0.79	0.4343
Boldness	0.04	0.3969	0.09	0.9294
Neophobia	0.23	0.4091	0.56	0.5771
After variable select.; adj r^2 = 0.147				
Constant	0.42	0.2135	1.97	0.1132
Urbanization (U)	-0.86	0.3073	-2.80	***0.0426***

Table S4. Models with acquisition Learning (trials) as the response variable

Linear model (LM)

Full model; adj r^2 = -0.124	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	0.746	1.255	0.59	0.5569
Urbanization (U)	-0.035	0.095	-0.36	0.7180
Tarsus length	0.034	0.056	0.60	0.5528
Sex (M)	-0.041	0.081	-0.51	0.6164
Body weight	-0.027	0.026	-1.05	0.3037
Body condition	1.387	0.955	1.45	0.1570
Boldness	0.093	0.081	1.15	0.2611
Neophobia	0.035	0.072	0.49	0.6251
After variable select.				
N/A				

Linear mixed model (LMM) with site as a random factor

Full model; adj r^2 = -0.137	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	0.660	1.357	0.49	0.6308
Urbanization	-0.032	0.090	-0.36	0.7278
Tarsus length	0.028	0.060	0.46	0.6461
Sex	-0.038	0.092	-0.41	0.6835
Body weight	-0.015	0.028	-0.55	0.6003
Body condition	1.366	1.042	1.31	0.2002
Boldness	0.092	0.090	1.02	0.3167
Neophobia	0.044	0.080	0.55	0.5842
After variable select.				
N/A				

Table S5. Models with reversal Learning (trials) as the response variable

Linear model (LM)

Full model; adj r^2 = 0.018	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	1.798	1.516	1.19	0.2459
Urbanization (U)	-0.044	0.124	-0.35	0.7264
Tarsus length	-0.048	0.069	-0.70	0.4892
Sex (M)	-0.130	0.102	-1.27	0.2142
Body weight	0.049	0.031	1.55	0.1327
Body condition	0.711	1.153	0.62	0.5430
Boldness	-0.055	0.102	-0.54	0.5953
Neophobia	-0.009	0.100	-0.09	0.9301
After variable select.				
N/A				

Linear mixed model (LMM) with site as a random factor

Full model; adj r^2 = -0.137	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	1.180	1.660	0.71	0.4834
Urbanization	-0.014	0.106	-0.13	0.8972
Tarsus length	-0.042	0.072	-0.59	0.5625
Sex	-0.083	0.116	-0.72	0.4823
Body weight	0.068	0.027	2.57	***0.0469***
Body condition	0.403	1.190	0.34	0.7377
Boldness	-0.006	0.110	-0.05	0.9600
Neophobia	0.011	0.107	0.10	0.9212
After variable select.				
N/A				

Table S6. Models with PHA response (mm) as the response variable

Linear model (LM)

Full model; adj r^2 = 0.381	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	-27.362	75.549	-0.36	0.7196
Urbanization (U)	20.231	4.563	4.43	***0.0001***
Tarsus length	3.508	3.324	1.06	0.2991
Sex (M)	-2.385	4.577	-0.52	0.6059
Body weight	-2.380	1.550	-1.54	0.1344
Body condition	-19.923	57.585	-0.35	0.7316
After variable select.; adj r^2 = 0.362				
Constant	11.933	2.469	4.83	< 0.0001
Urbanization (U)	18.867	3.703	5.10	***< 0.0001***

Linear mixed model (LMM) with site as a random factor

Full model; adj r^2 = 0.642	**Estimation**	**SE**	**t**	**Prob.>|t|**
Constant	-64.028	63.314	-1.01	0.3209
Urbanization	19.776	7.229	2.74	***0.0355***
Tarsus length	3.716	2.872	1.29	0.2063
Sex	-0.611	3.907	-0.16	0.8768
Body weight	-0.607	1.717	-0.35	0.7262
Body condition	-15.428	49.896	-0.31	0.7595
After variable select.; adj r^2 = 0.639				
Constant	11.809	4.671	2.53	0.0467
Urbanization (U)	17.875	6.685	2.67	***0.0369***

Table S7. Correlations between all variables along with urbanization, either as a fixed affect or interacting with response variables

Explanatory variables		**Response variable**					
		Boldness	Neophobia	Prob. Solv. PC1	Acq. Learn.	Rev. Learn.	Immunocomp.
Boldness (+Urb.)	r^2	-	0.208	0.165	0.028	0.005	0.384
	p	-	0.429	0.148	0.230	0.620	0.470
Boldness*Urb.	r^2	-	0.214	0.177	0.052	0.049	0.386
	p	-	0.601	0.413	0.277	0.157	0.737
Neophobia (+Urb.)	r^2	0.193	-	0.103	0.054	0.013	0.431
	p	0.429	-	0.842	0.253	0.486	0.502
Neophobia*Urb.	r^2	0.205	-	0.103	0.065	0.028	0.440
	p	0.441	-	0.999	0.494	0.448	0.413
Prob. Solv. PC1 (+Urb.)	r^2	0.191	0.219	-	0.005	0.000	0.376
	p	0.148	0.842	-	0.696	0.950	1.000
Prob. Solv. PC1*Urb.	r^2	0.245	0.219	-	0.006	0.000	0.377
	p	0.007	0.944	-	0.771	0.985	0.878
Acq. Learn. (+Urb.)	r^2	0.202	0.254	0.148	-	0.195	0.380
	p	0.095	0.164	0.683	-	***0.002***	0.645
Acq. Learn.*Urb.	r^2	0.205	0.261	0.148	-	0.196	0.382
	p	0.691	0.550	0.859	-	0.844	0.690
Rev. Learn. (+Urb.)	r^2	0.183	0.258	0.120	0.196	-	0.388
	p	0.620	0.486	0.899	***0.002***	-	0.351
Rev. Learn.*Urb.	r^2	0.218	0.271	0.200	0.196	-	0.391
	p	0.165	0.420	0.979	0.957	-	0.771
Immunocomp. (+Urb.)	r^2	0.124	0.227	0.118	0.011	0.023	-
	p	0.470	0.502	1.000	0.645	0.351	-
Immunocomp.*Urb.	r^2	0.189	0.170	0.119	0.011	0.031	-
	p	0.077	0.777	0.821	0.995	0.575	-

Connecting statement

In chapter 2, we showed for the first time that innovativeness increases with urbanization in a wild bird. We found that boldness and neophobia are also higher in urbanized areas. Unexpectedly, immunity is not traded-off with problem-solving but instead increases considerably with the urbanization gradient as well.

In addition to the two obstacle-removal problem-solving tasks used in chapter 2, we presented to the same birds the well-known string-pulling task. The results of those tests are presented in chapter 3. The string-pulling task is a complex problem that is sometimes thought to require insight. We hypothesize that performance on the string-pulling task should co-vary with performance on the two other problem-solving tasks that varied with urbanization, and perhaps with temperament. Surprisingly, the relation between string-pulling performance and other cognitive skills was never tested before.

Chapter 3.

Bajan birds pull strings: Two wild Antillean species enter the select club of string-pullers

Audet J.-N., Ducatez S. and Lefebvre L.

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Abstract

String-pulling is one of the most popular tests in animal cognition because of its apparent complexity, and of its potential to be applied to very different taxa. In birds, the basic procedure involves a food reward, suspended from a perch by a string, which can be reached by a series of coordinated pulling actions with the beak and holding actions of the pulled lengths of string with the foot. The taxonomic distribution of species that pass the test includes several corvids, parrots and parids, but in other families, data are much spottier and the number of individuals per species that succeed is often low. To date, the association between string-pulling ability and other cognitive traits was never tested. It is generally assumed that string-pulling is a complex form of problem-solving, suggesting that performance on string-pulling and other problem-solving tasks should be correlated. Here, we show that two innovative species from Barbados, the bullfinch *Loxigilla barbadensis* and the Carib grackle *Quiscalus lugubris fortirostris*, pass the string-pulling test. Eighteen of the 42 bullfinches tested succeeded, allowing us to correlate performance on this test to that on several other behavioral measurements. Surprisingly, string-pulling in bullfinches was unrelated to shyness, neophobia, problem-solving, discrimination and reversal learning performance. Only two of 31 grackles tested succeeded, precluding correlational analyses with other measures but still, the two successful birds largely differed in their other behavioral traits.

Introduction

String pulling behavior was first described nearly two millennia ago by Pliny the Elder, who observed captive goldfinches pulling buckets of water (see Jacobs & Osvath 2015). Since then, the string-pulling task and its numerous variations have been used on several taxa such as cats (Whitt et al. 2009), dogs (Osthaus et al. 2005) and apes (Völter & Call 2012). Still, cognitive studies involving birds dominate the string pulling literature (Jacobs & Osvath 2015). In birds, the paradigm involves retrieving a visible, out-of-reach reward by pulling a vertical string that is sufficiently long so that the bird has to pull sequentially several times while maintaining the pulled portions of the string with its foot. String pulling is considered one of the most complex problem-solving tasks and it has been proposed that "insight" (Heinrich 1995; Pepperberg 2004; Werdenich & Huber 2006) or "imagination" (Emery 2004) are required as the test is quickly passed by some animals without any apparent trial-and-error (Werdenich & Huber 2006). However, such explanations have been the focus of much debate and most researchers, on the basis of tests that disrupt direct string-reward connections, agree nowadays that animals use positive perceptual-motor feedback as a reinforcement to eventually complete the task (Vince 1961; Dücker & Rensch 1977; Osthaus et al. 2005; Seibt & Wickler 2006; Taylor et al. 2010; Taylor et al. 2012; Seed & Boogert 2013; Jacobs & Osvath 2015). Whatever the case may be, completing this task involves a degree of sequential coordination, as it requires successive actions that are not immediately rewarded, where a bird has to produce a coherent suite of pulling movements with its beak and holding loops of string with its feet.

Although the number of bird species shown to solve the string-pulling task is growing steadily, most of them belong to only a restricted set of families. Large-brained species from the parrot and Corvid clades make up the vast majority of them, while tests on several *Paridae* (tits) species also yield positive data (see Table 3 in Jacobs & Osvath 2015). Studies on other Oscine families reveal much more mixed results. The avian superfamily *Passeroidea* in particular, an extremely diverse and globally distributed clade, shows wide variation between species and individuals in success or failure at the test. Within *Fringillidae*, for example, the family that includes the best-studied species,

the Eurasian goldfinch, eight out of the ten species listed in (Jacobs & Osvath 2015) yield both positive and negative results. Outside of Corvids, Psittacids and Parids, there is thus extensive taxonomic and individual variation. Testing new species is important to obtain a coherent picture of taxonomic variation if we are to compare string-pulling to other, well-studied cognitive measures. In particular, associations between different cognitive measurements and performance at string pulling could provide information on the abilities required to succeed at this task. In addition, the string-pulling paradigm is one of a very few tasks that can be used across a wide variety of taxa.

Two other features of the avian string-pulling literature research are also noteworthy. First, most birds used in the studies were raised in captivity, be it in laboratories or zoos. Familiarity with (and often hand-raising by) humans might facilitate solving of the string problem, with, for example, conspecific tutoring (Seibt & Wickler 2006) or long periods of acclimatization (Ellison et al. 2015) sometimes included in the protocol. Notable exceptions are the work of Taylor and colleagues (Taylor et al. 2010; Taylor et al. 2012) on wild-caught crows *Corvus moneduloides* studied in aviaries in their native New Caledonia, as well as that of Millikan and Bowman (1967) on seven species of Darwin's finches. Second, several studies were done on a very small number of individuals per species or a number that is unspecified in the papers. Given the often low success rate obtained on species where large numbers of individuals are tested, false negatives might be frequent when species with small sample sizes fail the test.

In this paper, we test wild-caught individuals from two species not previously examined and attempt to correlate their string-pulling performance with their results on other tests. We show that wild-caught Barbados bullfinches *Loxigilla barbadensis* and Carib grackles *Quiscalus lugubris fortirostris*, two innovative Barbadian species (Lefebvre et al. 2016), pass the basic string-pulling test, and we ask if individual variation in performance can be predicted by results on other tasks. From previous experiments on the same individuals (Ducatez et al. 2015; Audet et al. 2016), we had data on shyness, neophobia, problem-solving, discrimination learning and reversal learning. The shyness test assessed an individual's latency to feed after being disturbed

by an experimenter, while neophobia measured a similar latency when a novel object was placed near the food. Two problem-solving tasks required novel motor acts to access visible food. In the discrimination-learning task, individuals needed to choose the correct color cue identifying a food container. In the reversal learning test, individuals needed to reverse their former association by inhibiting responses towards the formerly rewarded color, shift their attention and form a new association with the previously unrewarded color. Based on previous results on the same species, we predicted that string-pulling would correlate with the other problem-solving tasks, but would show either a negative or no correlation with discrimination learning and/or reversal scores (Ducatez et al. 2015; Audet et al. 2016). Several studies suggest that discrimination and reversal learning paradigms measure different abilities compared to problem-solving tasks (Griffin et al. 2013; Isden et al. 2013; Ducatez et al. 2015; Shaw et al. 2015; Audet et al. 2016).

Millikan and Bowman (1967) have previously tested species from the *Passeroidea* superfamily to which our grackles (family *Icteridae*) and Barbados bullfinches (family *Thraupidae*) belong. Neither of the two Icterids (Brewer's blackbird, *Euphagus cyanocephalus* and Red-winged blackbird, *Agelaius phoeniceus*) passed the test, nor did the only Thraupid that is not a Darwin's finch, the Cuban grassquit *Tiaris canora*, a close relative of the Barbados bullfinch (Burns et al. 2014). Given that the number of individuals tested per species is not mentioned in Millikan and Bowman (1967), these results could be false negatives if sample sizes were small. Here, we test a total of 73 wild-caught birds and show that individuals from our two species can pass the string-pulling test.

Methods

Subjects. All birds were captured between February and April 2013 and were kept in individual cages at the Bellairs Research Institute, St. James, Barbados. Details on captures and housing conditions are given in Audet et al. (2016), Ducatez et al. (2015), and in the S1 Appendix. Briefly, forty-two Barbados bullfinches were caught at eight different sites on the island of Barbados, and thirty-one Carib grackles on the grounds of the Bellairs Research Institute of McGill University, St. James, Barbados. All birds

were released at their initial site of capture at the end of our experiments. All our procedures were approved by the McGill University Animal Care Committee (Animal Use Protocol 2013-7140) as well as the Natural Heritage Department of the Barbados Ministry of Environment and Drainage (permit 8434/56).

Experimental procedures. The string-pulling task featured a transparent cylindrical container (height: 3 cm, diameter: 3 cm) in which a food reward (finch seed mix for bullfinches, soaked dog pellets for grackles) was suspended. This container was attached to a wooden perch using a 25 cm (bullfinches) or 50 cm (grackles) string that was suspended inside a transparent PVC cylinder (height: 60 cm, diameter: 6 cm) so that it prevented the birds from obtaining the reward by flying to it (see Figure 1). The task was presented for a maximum of 10 trials of 5 minutes each with 10 min between two trials, on the same day. A bird was considered successful if it pulled the container to its reach and fed from it. The string-pulling test was presented on the 7th day of captivity, without habituation to the task, and after all other behavioral tests were completed (see S1 Appendix). To test for potential improvement in performance, the task was presented again to solvers five minutes after their first success.

We compared performance of our 73 individuals on the string pulling test to data we obtained in previous experiments (see Ducatez et al. 2015; Audet et al. 2016 and S1 Appendix for details). Here, we briefly summarize the six tasks used in these experiments. All birds were given a two-day period of habituation to captivity after capture. On day 2 (grackles) or 3 (bullfinches), each individual's latency to feed following presentation of an open food dish by an experimenter was measured (shyness test). On day 3, latency to feed from an open dish was again measured, but this time with a novel object placed beside the feeding dish. We measured neophobia as the latency to feed in this test minus the latency to feed in the shyness trial with no novel object. Birds were then given 10 five min trials of a problem-solving task in which they had to flip a lid (grackles) or pull a lid or a drawer (bullfinches) on a transparent box that contained seeds (bullfinches) or a piece of soaked dog pellet (grackles). We started measuring the latency to succeed when an individual touched the apparatus for the first time, thus removing initial boldness or neophobia effects from the problem-solving latency. A

second problem-solving task, the tunnel task, was given on the next day. It consisted of a transparent rectangular box opened on only one side. A transparent cylindrical tube containing food was inserted at the closed end of the tunnel and a wooden stick was attached to it so that the birds had to pull on the stick to get the tube out of the tunnel. Birds were again given a maximum of 10 trials each lasting 5 min and problem-solving latency was measured in the same way as for the previous task. For bullfinches, discrimination learning was assessed with two petri dishes (same as the one used for shyness) each inserted in a wooden platform painted either green or yellow, open on one side, and placed at each extremity of the cage. The dish inside the unrewarded color contained seeds glued to the bottom of the dish, so that no difference could be seen from a distance but the seeds were impossible to remove for the birds (see Audet et al. 2016 and S1 Appendix for details). For grackles, the apparatus consisted of two lid-covered cylinders (same as the lid-flipping task) covered with different colors of tape. Contrary to the lid-flipping task, the birds could not detect the presence of food inside the cylinders because of the opaque tape, but could associate a color with the presence of a reward. The learning criterion was choice of the correct color on seven consecutive trials. Reversal learning on both tasks was assessed the following day by switching the rewarded color and measuring the number of trials to achieve the same success criterion as in the initial discrimination learning phase. Note that the reversal learning test on grackles was not described in Ducatez and colleagues (2015) but it is summarized here to compare with string-pulling performance.

Analyses. We first built models using all behavioral variables. Linear models were built using latency to succeed the string pulling as the dependent variable. Shyness and neophobia latencies, trials to criterion on the discrimination learning and reversal tasks, as well problem-solving latencies were used separately as explanatory variables. We ran two versions of these models, one with and one without non-solvers included in the analyses; in the former case, non-solvers were assigned the maximum latency plus one (3001 seconds).

We then built a second round of linear models that included all the aforementioned variables as well as body condition, weight, sex and capture site as

potential confounding variables in the models. We also conducted the analyses with success or failure at string pulling as a binary response variable instead of the latency to succeed, incorporating all explanatory variables mentioned above, using a binomial distribution and a logit link. Stepwise variable selection was achieved using all variables in single models (one model for solvers only and another that included non-solvers). JMP 11.0 software (SAS Institute, Cary, NC) was used to compute all linear models. The p-value threshold was determined using a Bonferroni correction according to the number of models conducted for each category.

Results

Eighteen out of the 42 bullfinches (43%) succeeded in completing the string-pulling task within 10 trials (mean = 4.2 trials, Std. Dev. = 2.8). Two out of the 31 tested grackles also completed the task (on the first and seventh trials). Typically, the birds that succeeded pulled the string a few times using only their beak, and then started to jointly use their beak and their foot to hold the string and bring the container closer to the perch they were standing on (Figure 1, S1 and S2 movies). For both species, 3 to 5 pull-grab movements were necessary in order to bring the container close enough to feed from it, and they used the exact same technique when successful. We presented the task a second time to solvers. All of them completed the task during the 5-minute trial following the first success. In bullfinches, mean latency was much lower for the second success than for the first (1^{st}: 1135 ± 203.3 s; 2^{nd}: 75.29 ± 16.49 s; $p_{\text{t-test}} < 0.001$). For the two successful grackles, one was, like the bullfinches, 15 times faster on its second success than on its first (1^{st}: 1914 s, 2^{nd}: 126 s). The second grackle did not significantly improve, however, possibly due to its very fast performance on its first success (1^{st}: 62 s, 2^{nd}: 83 s). Most bullfinches gradually learned to master the technique by trying several times to grab the string (Figure 1B and Figure 1C), then implementing grabs using a foot hold (Figure 1D and Figure 1E) and finally coordinating a sufficient number of the latter to master the task (Figure 1F; see S2 Figure for a summary of the mean number of trials required for each movement).

In bullfinches, the latency to succeed was not affected by shyness (solvers: $r^2 = 0.007$, $p = 0.7533$; all animals: $r^2 = 0.046$, $p = 0.1789$, S1 Figure A) or neophobia

(solvers: $r^2 = 0.003$, $p = 0.8277$; all animals: $r^2 = 0.011$, $p = 0.5379$, S1 Figure B). Problem-solving scores on the two other tasks performed by the same animals (see (Audet et al. 2016)) were not associated with string-pulling scores, whether the non-solvers were included in the analysis or not (Tunnel task: solvers: $r^2 = 0.034$, $p = 0.6360$, all animals: $r^2 = 0.005$, $p = 0.6691$, S1 Figure C; Lid-Drawer task: solvers: $r^2 = 0.043$, $p = 0.4068$, all animals: $r^2 = 0.024$, $p = 0.3267$, S1 Figure D). Likewise, string-pulling was not associated with discrimination learning (solvers: $r^2 = 0.001$, $p = 0.8850$, all animals: $r^2 = 0.047$, $p = 0.1694$, S1 Figure E) or reversal learning scores among solvers ($r^2 = 0.163$, $p = 0.0962$, S1 Figure F, solid line). There was a significant negative correlation between latency to succeed and reversal learning scores when non-solvers were included in the analysis ($r^2 = 0.146$, $p = 0.0125$, S1 Figure F, dashed line), but with a Bonferroni correction, the result was non-significant ($n_{TESTS} = 10$; $\alpha = 0.005$). Including sex, body condition and capture site in the previous models did not change any of the results, and these new variables were not significantly associated with string-pulling scores. Building a linear model with success or failure as a response variable and all the aforementioned variables did not yield any significant predictor of success at string pulling following stepwise selection.

Given the low number of successful grackles (2 out of 31), we could not build linear models for this species. The two successful individuals differed sharply in their responses to the behavioral and cognitive tests. One of them was particularly shy and did not eat during the shyness trial. This same individual did not eat either during the neophobia trial. It did not solve any of the problem-solving tasks, but, in contrast, it ranked 6th on the discrimination learning task, reaching the success criterion after 17 trials (mean number of trials for the 31 individuals = 34.22 ± 3.54), and ranked 13th for reversal learning, reaching the success criterion after 47 trials (mean number of trials for the 31 individuals = 56.23 ± 4.44). The second individual behaved very differently: it was relatively bold (it ate after 245 seconds at the shyness trial, ranking 14th), not neophobic (it ate after 4 seconds on the neophobia trial, ranking 2nd); this individual also solved the two problem-solving tasks (after 2122 s for lid-flipping, as compared to an average of 2165 ± 204 s for the 31 individuals, and 2748 s for the tunnel, compared to an average of 2566 ± 509 s for the 31 individuals). In contrast, it ranked 26th out of 31

individuals at discrimination learning (succeeding after 52 trials), and 19th at reversal learning (succeeding after 54 trials).

Discussion

Our study provides new information on two species that differ substantially in their ability to acquire the string pulling task. The strong performance of bullfinches is in line with their particularly high innovativeness in Barbados (Lefebvre et al. 2016). The Barbados bullfinch is a close relative of Darwin's finches and belongs to a family, *Thraupidae*, that shows a high propensity for innovative behaviors (Lefebvre et al. 2016). The fact that the entire clade of Darwin's finches seems to be innovative led Tebbich and colleagues (2010) to propose a version of West-Eberhard's (2003) flexible stem hypothesis. Tebbich and colleagues found that woodpecker finch physical cognition did not differ from that of non-tool using Galapagos finches, suggesting that innovativeness could have played a role in the whole ancestral clade's ability to colonize new environments and diversify. The string-pulling literature suggest a similar clade-level effect for *Thraupidae*: Millikan and Bowman (1967) concluded that the string-pulling success of woodpecker finches was not superior to that of the four other successful, but non-tool using Galapagos finches or of the tufted titmice the study also tested. The strong performance of Barbados bullfinches further suggests that Thraupid cognitive performance is a general property of the whole clade. Environmental conditions in Barbados (low level of predation and competition, limited resources due to small island size, intense anthropogenic modification of the original environment) are also likely to facilitate the emergence of innovative behaviors. Interestingly, the negative results of Millikan and Bowman (1967) on the Cuban grassquit (another Thraupid species), if they are not a false negative due to small sample size, parallel the poor performance on other cognitive tests of the sister species of *T. canora* in Barbados, the black-faced grassquit *T. bicolor* (Kayello 2013; Lefebvre et al. 2016).

If the strong performance of Barbados bullfinches here is in line with the generally positive results (five out of six Galapagos Darwin's finch species tested) that Millikan and Bowman (1967) obtained on *Thraupidae*, the much lower numbers of successful Carib grackles also fit with Millikan and Bowman's negative results on the

two Icterid species they tested. Given that the number of birds per species that they tested is unspecified in Millikan and Bowman's (1967) paper, it is impossible to say whether a large sample of Brewer's blackbird and Red-winged blackbird could have revealed a low, but nonetheless non-zero number of string-pulling success in these species as it did in Carib grackles. It is ironic that given our much weaker results on grackles compared to bullfinches, one of the very few anecdotal observations of string-pulling in the wild is on the Carib grackle's sister species, the Greater-Antillean grackle *Q. niger*. Graves (2006) describes how a grackle used a sequence of beak pulling and foot holding actions to pull up the hind section of a dead anole that was dangling on a piece of skin.

For the first time, to our knowledge, individual variation in string pulling performance was compared to six other behavioral measurements that included temperament, learning and problem-solving. However, despite the diversity of tasks considered in our study, we were not able to link string-pulling performance with either shyness, neophobia, problem-solving or discrimination and reversal learning performance. The only association we found was a *negative* one between reversal learning and string-pulling in bullfinches, but this result was not significant after correction for our large number of comparisons, and was mostly due to a potential outlier (see S1 Figure F). In any case, this association was weak, confirming the low, if not inexistent, correlation of performance at string-pulling with other measures. This result was particularly robust in bullfinches, where 18 out of 42 birds succeeded at the string-pulling task, allowing us to run linear analyses. In grackles, only two birds succeeded, precluding statistical analysis, but they were positioned at opposite ends of the speed-accuracy continuum identified in previous work (Ducatez et al. 2015). One grackle was bold and not neophobic, fast at problem-solving, but made many learning errors, while the other showed the opposite pattern. These results confirm that, similar to the situation in bullfinches, performance at string-pulling is not correlated with other measures in grackles.

Our results suggest that the string-pulling task may involve skills that differ from the mix of memory, motivation, motor diversity and/or persistence that affect performance in problem-solving and in discrimination and reversal learning. Physical cognition, and potentially causal reasoning, might be necessary to succeed at this task,

as individuals need to organize behaviours requiring a high number of sequential and coordinated operations. Comparing performance on string-pulling and other physical cognition tasks, such as the cane (e.g. Teschke et al. 2011; Teschke et al. 2013), trap-tube (Taylor et al. 2009) or water displacement tasks (Bird & Emery 2009), might provide important information on the cognitive skills required to solve the string-pulling task. Differences in physical cognition and causal reasoning abilities might explain the differences in performance we obtained between bullfinches and grackles, in a way problem-solving, discrimination and reversal learning tests could not.

Even if a few of our birds succeeded on the very first trial (two bullfinches and one grackle), most solvers required several trials in which they tried different strategies that gradually led to success (S2 Fig). Furthermore, there was a significant improvement when the task was given a second time to the solvers: latency decreased 15-fold in bullfinches and one of the two successful grackles. Taken together, these data suggest that motor trial-and-error learning was occurring, at least in some individuals. The gradual improvement leading to success over trials and the latency reduction following repeated successes is also seen in the majority of other string-pulling investigations in birds (Dücker & Rensch 1977; Schuck-Paim et al. 2009; Taylor et al. 2010; Krasheninnikova 2013; Ellison et al. 2015). Because our birds were wild-caught, we cannot exclude that differences in individual experience in the field might have affected performance, facilitating for instance the rapid success of the birds that solved in their first trials.

While recent research has focused on modifications of the basic string-pulling procedure we used here to pinpoint the animal's understanding of the task (Heinrich & Bugnyar 2005; Taylor et al. 2010; Bagotskaya et al. 2012), there has been little work on the relationship between string-pulling and other behavioral and cognitive variables. The fact that we did not detect any correlations in our study is puzzling, despite the fact that previous work on Barbados bullfinches and Carib grackles has found coherent relationships between the measures we tested against string-pulling (Ducatez et al. 2015; Audet et al. 2016). It is possible that string pulling is associated with traits we did not measure, but Millikan and Bowman (Millikan & Bowman 1967) had already expressed

surprise that tool-using woodpecker finches did not differ from non-tool using Darwin's finches in their ability to solve the test.

One important feature of current research is the use of task variants that manipulate the perceptual and causal cues between the string and the reward (Heinrich & Bugnyar 2005; Taylor et al. 2010; Bagotskaya et al. 2012). For example, Taylor and colleagues (2010) have found that the New Caledonian crow's performance was greatly reduced when a platform was used to eliminate the visual feedback of the meat getting closer to the animal. When the same apparatus was employed with a mirror that provided visual feedback, performance was comparable to that of the basic string-pulling paradigm, suggesting that operant conditioning, rather than insight, is the mechanism enabling the birds to master this task. Similarly, when the string is attached to a pulley so that the birds need to pull down in order to get the suspended reward, which is counter-intuitive for the birds, the success rate is also significantly reduced (Heinrich & Bugnyar 2005). We do not deal with these issues in our study, so we cannot infer the level of physical cognition involved in our birds' success. The next step would obviously be to use similar protocols with Barbados bullfinches in order to disentangle which mechanisms lead to the high string-pulling success in this species.

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Figure

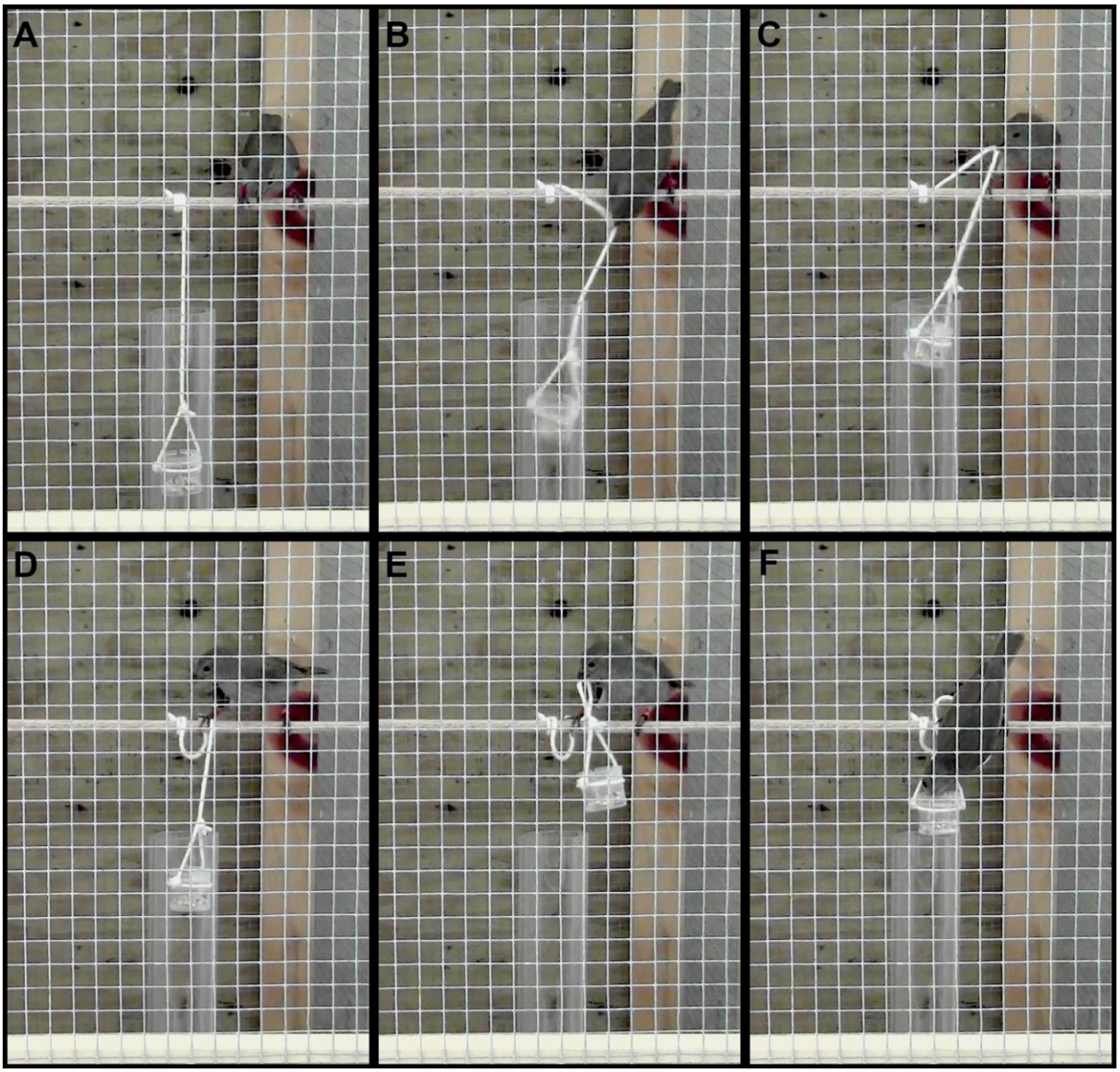

Figure 1. Typical sequence performed by bullfinches to master the string pulling task. A) The bird is on the perch where the string is attached and B) grabs the string to C) lift the plastic container. D) The string is maintained in place with the leg and E) the procedure is repeated with lift-grab combinations until F) the container is reachable.

Supporting Information

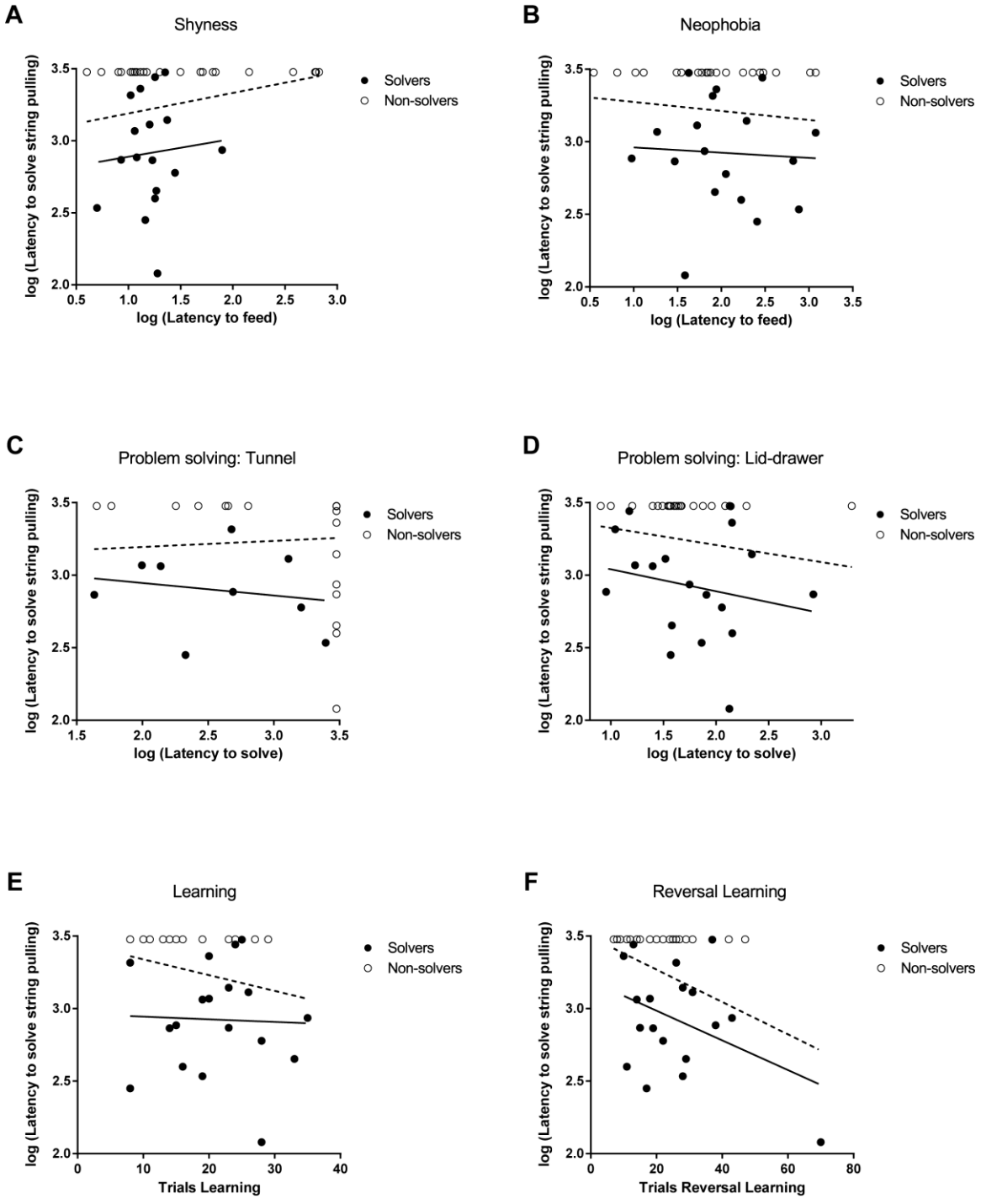

Figure S1. Linear regressions between string pulling performance and all other measured behavioral traits. String-pulling latency vs A-B: Shyness and neophobia, C-D: Problem-solving, E-F: Discrimination and reversal learning. Linear regressions with all animals including non-solvers on the string pulling task (which were attributed the maximum latency +1: 3001 s) are represented by dashed lines whereas filled lines are linear regressions in which non-solvers were removed.

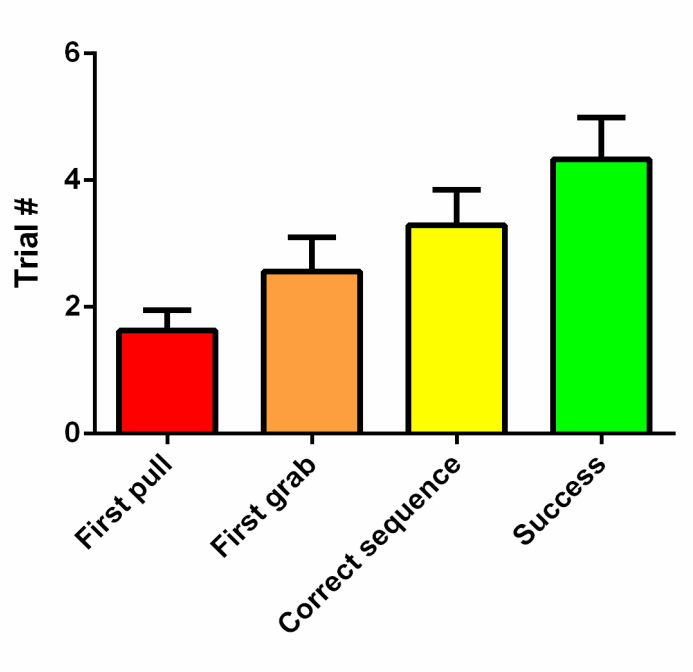

Figure S2. String-pulling success progression in Barbados bullfinches. Average number of trials needed for Barbados bullfinches to reach every major step of the string pulling task. Bars represent means ±SEM.

S1 Movie. Barbados bullfinch performing string-pulling: https://youtu.be/CD_CDx0vZr8

S2 Movie. Carib grackle performing string-pulling:

https://youtu.be/IxxwAEtaVbM

Table S1. Individual string pulling data.

		String pulling performance				
Number	**Sex**	**Trials**	**Latency (s)**	**Log (latency)**	**Success/Fail**	**2nd Latency (s)**
A12.O	F	2	342	2.5340	S	62
A14.O	F	5	1395	3.1446	S	59
A16.O	M	11	3001	3.4773	F	NA
A17.O	M	11	3001	3.4773	F	NA
A5.V	M	2	600	2.7782	S	NA
B10.V	M	3	767	2.8848	S	33
B11.V	M	3	732	2.8645	S	30
B12.O	F	11	3001	3.4773	F	NA
B12.V	M	11	3001	3.4773	F	NA
B13.V	M	10	2990	3.4757	S	60
B14.V	F	11	3001	3.4773	F	NA
B15.O	M	1	282	2.4502	S	110
B15.V	F	11	3001	3.4773	F	NA
B16.O	F	11	3001	3.4773	F	NA
B16.V	F	11	3001	3.4773	F	NA
B17.O	M	2	450	2.6532	S	8
B17.V	F	3	862	2.9355	S	105
B18.O	M	4	1154	3.0622	S	15
B18.V	F	11	3001	3.4773	F	NA
B19.O	M	4	1170	3.0682	S	60
B19.V	M	11	3001	3.4773	F	NA
B2.V	M	5	1296	3.1126	S	120
B20.O	M	11	3001	3.4773	F	NA
B23.O	F	11	3001	3.4773	F	NA
B24.O	F	11	3001	3.4773	F	NA
B25.O	F	10	2767	3.4420	S	301
B26.O	F	11	3001	3.4773	F	NA
B27.O	M	11	3001	3.4773	F	NA
B28.O	F	8	2300	3.3617	S	51
B29.O	F	2	398	2.5999	S	119
B3.V	F	11	3001	3.4773	F	NA
B30.O	M	11	3001	3.4773	F	NA
B4.V	M	1	120	2.0792	S	50
B6.V	M	11	3001	3.4773	F	NA
B7.O	F	11	3001	3.4773	F	NA
B7.V	M	11	3001	3.4773	F	NA
B8.O	NA	11	3001	3.4773	F	NA
B8.V	F	11	3001	3.4773	F	NA
B9.V	F	11	3001	3.4773	F	NA
P5.O	M	7	2070	3.3160	S	75
P6.V	F	11	3001	3.4773	F	NA
SP1.V	M	3	737	2.8675	S	22

Appendix S1. Supplementary methods.

Capture and sexing. Barbados bullfinches were caught using mist nets (see Audet et al. 2016 for details) and Carib grackles using baited (with dog pellets) walk-in traps (1 * 0.55 * 0.55 m; see details in Ducatez et al. 2015). All birds were visually but not acoustically isolated from each other (see Audet et al. 2016 and Ducatez et al. 2015 for more details on captivity conditions). Grackles were sexed based on morphological and behavioral observations, see Overington et al. (2011). Bullfinches were sexed with PCR using blood samples (Audet et al. 2014).

Behavioral tasks. The behavioral tasks were different for bullfinches and grackles, as we initially investigated the two species for independent projects with slightly different objectives. In bullfinches, problem-solving abilities were first assessed using a lid-drawer task which consisted of a 2 cm x 3 cm x 3 cm drawer made of white plastic constructed with a circular opening (1.5 cm diameter) at the top covered with a lid to which a hook was attached (Figure S2C). The birds had the opportunity to gain access to food by opening the lid or by pulling the drawer. Birds were given a maximum of 15 trials each lasting 5 min. The problem solving score was defined as the latency to succeed after the individual touched the apparatus for the first time. The second problem-solving task, the tunnel task, consisted of a transparent rectangular box (H: 3 cm x W: 3 cm x L: 10 cm) opened on only one side (Figure S2E, Movie S2), and is described in the main text. A tube had to be extracted from the tunnel, and the bird then had to remove the lid to gain access to seeds. Birds were given a maximum of 15 trials each lasting 5 min and problem-solving latency was measured in the same way as for the lid-drawer task.

In grackles, the first problem-solving task (hereafter called lid-flipping task) consisted of a transparent PVC cylinder (diameter = 3 cm, height = 5 cm), set in the middle of a petri dish, over which we placed a white plastic lid (3.4 cm diameter). The visible food reward (1/6 soaked dog pellet) was placed inside the cylinder. To solve the task, a bird had to flip the lid off the cylinder to gain access to the food (see Ducatez et al. 2015). The second problem-solving task (similar to the tunnel task used with bullfinches) consisted of a semi-transparent PVC ‘tunnel’ box open on one end (13 * 4 * 3.5 cm, see

Ducatez et al. 2015). An uncovered transparent cylinder (2 cm diameter, 3 cm height) containing the food reward was glued to the end of a wooden stick (17 X 1 cm) equipped with a metal O-ring (which acted as a handle) on the other end. This stick assembly was introduced inside the tunnel so that the transparent cylinder touched the closed end of the tunnel. To solve the task, a bird had to pull the wooden stick out of the tunnel to gain access to the reward. For both tasks, a bird accessing food once was considered as successful, and each individual was allowed a maximum of 10 trials of 5 min each with 10 min between two trials per task.

Associative learning was assessed using a standard color discrimination task, in which the birds had to associate a given color with a food reward. For bullfinches, the test apparatus consisted of two Petri dishes, each inserted in a wooden platform (10 x 10 x 10 cm) painted either green or yellow and open on one side (see main text and Audet et al. 2016). For grackles, the apparatus consisted of two lid-covered cylinders (as in the lid-flipping task) covered with different colors of tape. Note that immediately after the last obstacle-removal trial, we eliminated initial individual differences in motor performance by shaping all birds to the same fast, reliable level of lid-flipping before testing started (see Ducatez et al. 2015 for details). A "color bias" trial was first conducted, where the bird was allowed to eat from one dish/cylinder, and the color chosen by the bird was considered its preferred color. The other color thus became the rewarded one in order to control for initial color bias. The bird was given up to 5 min to choose a dish. If the bird chose the rewarded color, it was allowed to feed (15 sec. for bullfinches, 1/6 soaked dog pellet for grackles). If the bird chose the unrewarded color, the two apparatuses were immediately removed by the experimenter. The location of the rewarded apparatus was switched at each trial to control for spatial preference. The learning criterion was choice of the correct color on seven consecutive trials (Boogert et al. 2010). On the next day, the rewarded color was switched and became the non-rewarded color. The same criterion as in the acquisition learning phase was used to establish success. On the first reversal learning trial, all birds initially chose the previously rewarded color (which was incorrect at this stage), indicating that they effectively learned the color stimuli, and not a potential perceptible difference in the Petri dishes.

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Connecting statement

In chapters 1 to 3, we characterized in *L. barbadensis* the appearance of an innovation in the field, the link between problem-solving and urbanization and the relationship between different tests of cognition in captivity. In chapter 3, we showed that string-puling performance in *L. barbadensis* does not correlate with performance on other tasks that measure innovativeness. It is unclear if string-pulling involves a specific set of abilities (e.g. the physical cognition hypothesis), but it seems that it requires different skills compared to obstacle-removal. Since the latter problems potentially better represent innovativeness in the wild (they were specifically designed to mimic wild innovations), we decided to focus on this type of problem for our next and final investigation aimed at finding the neural correlates of innovation.

The objective of chapter 4 is to examine the characterization of innovation at a molecular level. For that aim, we compare *L. barbadensis* with a non-innovative sister species: *Tiaris bicolor*. The previous chapters, along with a growing body of literature on the subject, suggest that obstacle-removal tasks should accurately assess differences in innovation skills between the two sister species. We compare the brains of both species, first using a broad approach to comparing general patterns of gene expression in different brain regions, and then we focus more precisely on specific genes that could be responsible for divergence in innovation.

Chapter 4.

Divergence in problem solving and expression of glutamate receptors in wild finches

Audet J.-N., Kayello L., Ducatez S., Perillo S., Cauchard L., Howard J.T., O'Connell L.A., Jarvis E.D., Lefebvre L.

Abstract

Problem-solving and innovation are key components of intelligence. Here, we compare wild-caught individuals from two species that are close relatives of Darwin's finches, the innovative, opportunistic *Loxigilla barbadensis*, and its closest conservative sister species in Barbados, *Tiaris bicolor*. We found an all-or-none difference in their problem-solving skills. RNA-Seq analyses revealed interspecific differences in genes related to neuronal and synaptic plasticity in the associative pallium, especially in the nidopallium caudolaterale (NCL), a structure functionally equivalent to the mammalian prefrontal cortex. At a finer scale, we discovered robust differences in NMDA and metabotropic glutamate receptors expression between the species. In particular, the GRIN2B:GRIN2A ratio was considerably higher in the innovative *L. barbadensis*, both at the level of mRNA and protein, suggesting that divergence in avian intelligence is associated with similar neuronal mechanisms to that of mammals, including humans.

Introduction

Innovative problem solving is a key feature of intelligence and has played a major role in the evolution of both human and non-human animals. Growing evidence indicates that fundamental differences in innovative capacity and tool-use reflect the enlargement of certain brain structures, notably the neocortex in primates and the associative pallium in birds (Timmermans et al. 2000; Reader and Laland 2002; Mehlhorn et al. 2010). Beyond these differences in brain structure, however, we know very little about the processes that control divergence in innovative problem solving at the neuronal level. In birds in particular, innovation has been well-studied but detailed neurobiological investigations explaining natural variation in innovative problem-solving are still lacking despite repeated demands for such studies (Mace et al. 1980; Healy and Rowe 2007). The outstanding cognitive capacities of birds, comparable to those of primates in terms of innovation and tool-use (Emery and Clayton 2004), have been partly explained by similar neuronal densities in associative areas of both taxa (Olkowicz et al. 2016). Now, for the first time, we characterize divergence in innovative problem-solving at the level of gene expression, using state-of-the-art molecular techniques, including next-generation transcriptome sequencing (RNA-Seq).

Here, we compared two sister species of birds that show extreme differences in foraging strategies despite being sympatric: the innovative Barbados bullfinch *Loxigilla barbadensis* and the conservative black-faced grassquit *Tiaris bicolor*. We used a battery of cognitive tasks on wild-caught birds to first document the differences and similarities in innovative problem-solving and learning between the two species. We demonstrated that the differences in innovation in the field are matched by differences in problem-solving abilities with captive birds. We then found that the two species have different levels of gene expression in the area of their brain that is the avian equivalent of the cortex of mammals. In particular, differences in gene expression are highest in the nidopallium caudolaterale (NCL), a brain area that is functionally similar to the mammalian prefrontal cortex (Güntürkün 2005), where the genes that are upregulated in the innovative bullfinch specifically promote neuronal activity. By linking the striking

behavioral differences between two closely-related species with levels of gene expression, we have identified key components for the evolution of problem-solving.

The two studied species are close relatives of Darwin's finches and belong to the family *Thraupidae* (**Fig. 1A,** Burns et al. 2014), a neotropical clade that shows high rates of evolutionary diversification, colonization and feeding innovations in the wild (Lefebvre et al. 2016). In Barbados, the endemic *L. barbadensis* (**Fig. 1B**) has recently evolved from the Lesser-Antillean bullfinch *L. noctis* (Buckley and Buckley 2004) and frequently uses opportunistic, innovative feeding behaviors that take advantage of anthropogenic foods (Reader et al. 2002; Ducatez et al. 2013). In sharp contrast, *T. bicolor* (**Fig. 1C**) is conservative, shy and feeds on grass seed (Evans 2009). The two species are each other's closest relative in Barbados (**Fig. 1A**), where they overlap in their habitat use and they are both territorial.

Results

We captured adults of both species in mist nets in Barbados, housed them in aviaries, and presented them with a battery of problem solving, learning and boldness tests. The first test was an obstacle-removal problem designed to mimic technical innovations in the wild (**Fig. S1A**) (Griffin and Guez 2014). Consistent with their divergence in innovativeness in the field, we found an all-or-none difference in problem solving between the two species; 24 out of the 29 tested *L. barbadensis* completed the obstacle-removal task in a mean of 4.4 ± 1.09 trials, but none of the 15 *T. bicolor* tested succeeded before the maximum number of 15 trials allowed (**Fig. 1D**). The poor performance of *T. bicolor* was not due to lack of motivation: all individuals contacted the apparatus and the amount of time spent trying to solve it did not significantly differ between the two species (**Fig. S2A**). To eliminate the possibility that *T. bicolor,* which is smaller than *L. barbadensis*, was physically incapable of solving the task, we trained all the unsuccessful birds of both species for up to 60 trials (shaping procedure, see **SI text** and **Fig. S2C**). With our shaping, *T. bicolor* eventually solved the problem (**Fig. S2D**), albeit in 12 times more trials than the five *L. barbadensis* that required shaping (**Fig. S2B, E**).

The finding that *L. barbadensis* are better problem solvers than *T. bicolor* was further confirmed with a detour-reaching task (**Fig. S1B**). This test measures the ability

to inhibit a behavior (direct reach) in presence of an obstacle, forcing the individual to shift to an alternative strategy to obtain a reward (food, in our case). Again, *L. barbadensis* outperformed *T. bicolor*: they needed a lower number of trials to reach the success criterion (**Fig. 1E**).

The two species also differed in two types of novelty responses linked to innovativeness, namely neophobia and boldness. In independent tests, *L. barbadensis* tended to be bolder (**Fig. S3A**) and less neophobic (**Fig. S3B**) than *T. bicolor* (see **Table S1** for all significant linear models using behavioral variables). In contrast, the two species did not differ in two tasks that involve stimulus learning (**Fig. S1C**): color discrimination learning (**Fig. S3C**) and reversal learning (**Fig. S3D**). The two closely-related Thraupids are thus highly divergent for innovative problem-solving but they are similar in terms of habitat preference, territoriality and stimulus learning, yielding a clear behavioral basis for brain and genetic comparisons.

In birds, species differences in innovativeness have been found to be positively associated with allometrically corrected differences in the size of the whole brain and, more specifically, with associative areas (mesopallium and nidopallium) analogous to the mammalian neocortex (Timmermans et al. 2000; Overington et al. 2009; Wang et al. 2010; Jarvis et al. 2013; Pfenning et al. 2014; Sayol et al. 2016). Differences in problem-solving between *L. barbadensis* and *T. bicolor* were not reflected in differences in residual brain mass when plotted with other *Thraupidae* (**Fig. S4**). We therefore went to a deeper level, using molecular approaches to examine in progressively finer detail the expression levels of receptors in six areas of the brain: the associative pallium (mesopallium and nidopallium, including NCL), the motor arcopallium (tertiary pallium), visual entopallium (a part of the primary pallium), and the spatial hippocampus (**Fig. 2A**).

We first aimed at identifying all the genes that differed between *L. barbadensis* and *T. bicolor* in the six areas. We dissected these pallial cell populations in brain sections under a dissecting microscope, mRNA was extracted, cDNA libraries prepared and sequenced on 8 naïve (i.e. different from the ones tested above) individuals per species (see **SI text** for details). The resultant ~19.2 billion reads (~200 million paired-

end reads per sample) were mapped to the chicken (*Gallus gallus*) genome to obtain a non-biased differential gene expression analysis. Principal component analysis (PCA) of the mean expression pattern for all genes in each region per species revealed that PC1 explains most of the variation between regions and PC2 between species (**Fig. 2B**). Consistent with previous findings that were obtained by analyzing the expression of 50 genes by in-situ hybridization in the brains of a model songbird, the zebra finch (Jarvis et al. 2013), the entopallium clustered furthest away from all the other pallial populations and regions forming the associative pallium (mesopallium and nidopallium including the NCL) clustered next to each other. Similarly, hierarchical cluster analysis revealed that the entopallium was the most distant region from the others while the populations forming the associative pallium were very similar to each other, and the arcopallium and hippocampus clustered together (**Fig. S5**) (Jarvis et al. 2013). These findings indicate that our approach was successful at revealing molecular relationships between brain regions.

We next asked if there were differences between species, and found in the PC2 that the species differed the least in the entopallium, but the most in the mesopallium, followed closely by the nidopallium and the NCL within the nidopallium (**Fig. 2B**). This was concordant with the total number of differentially expressed genes, with the mesopallium, nidopallium and NCL showing the highest number of differentially expressed genes (**Fig. S6**). Normalizing with the total number of genes expressed in each region resulted in the same rankings, which means that the observed differences are not simply due to differences in the number of expressed genes per region (**Fig. S7A**).

Because of the similarity in their expression and their potential cooperative role in problem-solving, we combined the mesopallium and the nidopallium (including the NCL) to perform gene ontology (GO) analyses. GO clustering revealed an overrepresentation of differentially expressed genes related to synaptic signaling and localization, and to a lesser extent, myelination (**Fig. 2C**; see **Table S2** for all clusters). Genes upregulated in *L. barbadensis* were enriched for functions of neurogenesis and axonogenesis, as well as cellular communication and signaling (terms associated with neurotransmission; **Fig. 2C**; **Table S3** for all clusters). Genes upregulated in *T. bicolor*

were enriched in nucleoside metabolic process, apoptosis and ion transmembrane transport (**Fig. 2C**; see **Table S4** for all clusters). Overall, genes related to specific neuronal and synaptic functions appear to be the most represented in differentially expressed genes between the species.

Focusing on the genes that contain "*neuron*" or "*synap*" in any of their GO terms, *L. barbadensis* had more upregulated genes in the associative pallium than *T. bicolor* ($P\chi^2$ = 0.0075) (**Fig. 2D**). Of all the brain regions examined, NCL had the most upregulated genes in *L. barbadensis* compared to *T. bicolor* ($P\chi^2$ = 0.0031) (**Fig. 2E**). Selecting instead other sets of genes, for example genes that have "*apopto*" or "*mitochondri*" in their GO terms yielded no difference, or differences in favor of *T. bicolor*, in the number of upregulated genes in the two species (**Fig. S7B-E**). Together, those results suggest that divergence in problem-solving skills is associated with upregulation of genes in the associative pallium, and to a greater extent in the NCL, and that those differences are specifically related to neuronal and synaptic activity. This is in accordance with several lines of evidence that suggest that the nidopallium and mesopallium perform similar functions to upper layers of the mammalian cortex and that the NCL is involved in higher sensory processing and associative functions (Rehkämper et al. 1991; Güntürkün 2005).

To further gain insight into which groups of genes co-vary between species, we performed a co-expression network analysis, based on hierarchical clustering, and discovered 9 network modules that are significantly associated with species in the associative pallium (**Fig. S8**). Two modules consisted of genes enriched in synapse and adult behavior (**Fig. 2F**; for all other modules see **Fig. S9** and for all GO clusters per module see **Table S5**). Interestingly, the NMDA glutamate receptor subunits GRIN1 and GRIN2B, known to promote synaptic plasticity (McEntee and Crook 1993; Myhrer 2003), are found in these modules (**Fig. 2F**). Another metabotropic glutamate receptor (GRM2) also involved in cognition (Marek 2010) was upregulated in *L. barbadensis* in a cluster of genes enriched for organelle organization (**Fig. S9**).

This led us to analyze the expression of 18 glutamate receptors that was detectable by RNA-Seq among the various brain regions. We found that four out of five

subunits of NMDA receptors and three of the five metabotropic receptors were differentially expressed in the associative pallium (**Fig. 3A**). The other receptors did not show significant differences.

To validate the RNA-Seq analyses and determine the specificity of the anatomical profile, we performed *in situ* hybridization on the brains of our two species with these genes (**Fig. 3B**). We confirmed that GRIN1 and GRIN2B were upregulated in the associative pallium of *L. barbadensis* while GRIN2A was upregulated in *T. bicolor* (**Fig. 3C**). GRM2 was also significantly higher in the associative pallium of *T. bicolor* than in *L. barbadensis*. Consistent with the RNA-Seq analyses, none of the AMPA or kainate types of glutamate receptors differed in expression between species (**Fig. 3B, C**). Overall, most of the same genes identified by RNA-Seq were also significantly differentially expressed using *in situ* hybridization data. GRIN3, GRM3 and GRM4 were differentially expressed in the RNA-Seq analysis, while differences in GRIN3 and GRM4 expression in the *in situ* hybridization were not significant anymore once Bonferroni corrections were applied. Nevertheless, when measuring all genes tested with both approaches, we found a robust correspondence (correlation of *P*-values: $r_{\text{Spearman}} = 0.648$, $P = 0.0008$). Details on the expression of all glutamate receptors in individual regions by RNA-Seq and *in situ* hybridization are given in **Tables S6** and **S7** respectively.

The above findings suggest that the upregulation of NMDA glutamate receptors in *L. barbadensis* relative to *T. bicolor*, together with the other genes present in their modules, might be relevant for divergence in problem-solving capacity. The finding of opposite differences in GRIN2A and GRIN2B expression is striking, as these are the most acknowledged types of glutamate receptors linked with learning, memory, and cognition, where they play opposite roles (Yashiro and Philpot 2008). In particular, the GRIN2B:GRIN2A ratio is positively associated with intensity of LTP and LTD, dendritic spine density and learning proficiency (Yashiro and Philpot 2008; Brigman et al. 2010; Cui et al. 2013). We therefore investigated these two NMDA receptors more closely in each region of the pallium, using the mRNA quantification data obtained from *in situ* hybridization (**Fig. S10A**) and RNA-Seq, as well as protein levels quantified

using immunohistochemistry performed with GRIN2A- and GRIN2B-specific antibodies (**Fig. S10B**). We then computed the GRIN2B:GRIN2A ratio using *in situ* hybridization data (**Fig. 4A**), immunohistochemistry data (**Fig. 4B**) and RNA-Seq data (**Fig. 4C**). There was a clear agreement in the results obtained from the three methods: the GRIN2B:GRIN2A ratio was reliably higher in *L. barbadensis* in all examined regions except the entopallium, in line with expectations from the literature given the behavioral differences between the two species.

Discussion

The all-or-none difference in problem-solving between *L. barbadensis* and *T. bicolor* we found here is more likely the result of species-level evolutionary divergence than the outcome of differential experience with anthropogenic foods. In a separate study (Audet et al. 2016), *L. barbadensis* from forested rural areas of Barbados were compared to *L. barbadensis* from the same urbanized areas we caught birds from in this study: urban *L. barbadensis* were significantly faster than rural ones in solving two obstacle removal problems. Nevertheless, rural *L. barbadensis* by far outperformed the *T. bicolor* we studied here. Surprisingly, *L. barbadensis* did not differ from *T. bicolor* in reversal learning, a task that is presumed to require complex cognitive skills. This result is in line with rural versus urban *L. barbadensis*, which differed in their problem-solving skills but not in their reversal learning ability (Audet et al. 2016), in addition to increasing evidence that suggest that reversal learning and problem-solving tasks measure different abilities (Audet and Lefebvre 2017). In any case, the lack of difference in associative learning tasks further suggest that the neural differences that we observed between the two species are specifically related to their divergent problem-solving skills. The only other trait for which the two Thraupids differ is their risk-taking behavior, but this is unlikely to be the main factor in the species divergence in neural properties since i) differences observed in the associative pallium, in particular the 'cognitive' NCL, are much greater overall than those observed in the 'fear-processing' arcopallium (Roth et al. 2012) ii) several of the genes we found to be associated with species divergence are genes for which we have previous knowledge of their cognitive role (e.g. glutamate receptors).

Because relative brain size is associated with innovation, and that similar neuron counts in primates and birds were hypothesized to be responsible for comparable cognitive skills in both clades, it would have been reasonable to predict that *L. barbadensis* would have a bigger brain than *T. bicolor*, assuming that they have matching relative neuron densities. However, our analysis revealed that their relative brain size did not differ. Increases in pallial volume, neuronal density and receptor expression thus appear to be different ways in which the information processing that underlies innovatve problem-solving can be increased.

Our study is the first to link divergence in behavioral innovation in the field, problem-solving in captivity and receptor expression levels in the brain. The receptors that are upregulated in *L. barbadensis* are those that promote neuronal activity (GRIN2B, GRIN1, and GRIN3), while the ones that are upregulated in *T. bicolor* are receptors that diminish it (GRIN2A, GRM2, GRM3, and GRM4). The association between GRIN2B:GRIN2A ratio and behavioral divergence between *L. barbadensis* and *T. bicolor* is particularly appealing, considering that: i) this ratio is one of the most promising candidates to explain variation in mammalian intelligence (Cui et al. 2013); ii) in songbirds this ratio changes in the song learning nuclei, and is thought to contribute to changes in the critical period for vocal learning as the animals become adults (Heinrich et al. 2002; Chakraborty et al. 2017); and iii) glutamate receptors are highly conserved (Wada et al. 2004, also see **Table S8**) and their functions are thought to be similar across species (Dingledine et al. 1999). Up to now, studies on GRIN2B:GRIN2A ratio variation have been based on experimental comparisons of transgenic or ageing rodents and normal versus neurologically diseased humans (Cui et al. 2013). Our finding of natural variation in the ratio in wild sister species with divergent foraging strategies provides an excellent opportunity to study the convergent evolution of innovative problem-solving, similar to that proposed for song learning in birds and speech in humans (Jarvis 2004). A next obvious step would be to confirm on other avian species, and eventually other vertebrate taxa, the trends found here on *L. barbadensis* and *T. bicolor*.

Methods

Animals. For behavioral analyses, 30 *L. barbadensis* of both sexes and 15 male *T. bicolor* were captured using mist nets between February and May in Holetown, Barbados. *T. bicolor* are sexually dichromatic; the monomorphic *L. barbadensis* were sexed molecularly from blood samples. Sex had no effect on our results. After capture, birds were brought to aviaries and housed in individual cages, visually but not acoustically isolated from each other. After a 2-day habituation period during which the birds were left undisturbed and fed *ad libitum*, they were food-deprived overnight and tested on the next morning.

Behavioral tests. On the third day of captivity, we assessed boldness by presenting a Petri dish (same as during habituation period) full of seeds, hiding behind a curtain and measuring the latency to feed following the disturbance. Birds were given a capped value of 1201s if they did not feed before the 20 minutes allotted. The obstacle-removal apparatus (see picture of the task, **Fig. S1A**) was then presented for the first time open and full of seeds to measure neophobia (latency to feed in the apparatus), to which we subtracted the boldness latency. Once the bird had fed from the open apparatus, the problem-solving trial began, with the lid closed but loosely fitted. Birds were given a maximum of 15 trials of 5 minutes to solve the problem, after which they were attributed a capped value of 16 trials. After this phase, unsuccessful birds were gradually trained (shaped) to solve the task (see **SI Text** for details and **Fig. S2C**) up to 60 trials. Then, birds were given the detour-reaching task (see picture of the task, **Fig. S1B**). They were first trained to reach a seed at the center of an opaque cylinder without pecking on the sides for 7 trials in a row, after which they were given a transparent cylinder and they had to perform with the same success criterion (see **SI Text** for details). The discrimination learning task consisted in two colored platforms in which Petri dishes were placed, one with the seeds available and the other with the seeds glued to the bottom of the dish so that there was no reward for choosing this dish, although glued vs non-glued seeds were impossible to distinguish from a distance. The success criterion was set at 7 trials in a row choosing the correct (rewarded) color. On the following day, reversal learning was assessed using the same protocol, but with the rewarded color switched with the previously un-rewarded color.

RNA-Seq. A separate cohort of birds was captured to prevent the modulation of brain mRNA caused by the stress of captivity and the experience of the behavioral tests. 8 birds per species were used for RNA-Seq. Birds were decapitated and brains were put in RNA-later. Brains were then sliced in thick (400 μm) sections and dissected under a dissecting microscope. RNA from the 6 individual brain regions of the 8 individuals for both species was then extracted separately (for a total of 96 samples) using a standard Trizol extraction procedure. mRNA was purified using the MicroPoly(A)Purist Kit (cat # AM1919, ThermoFisher, Waltham, MA USA), which binds polyA regions on cellulose spin columns. Library preparations were then performed a NEXTflex™ Directional RNA-Seq Kit (dUTP-Based) (cat # 5129-06, Bioo Scientific, Austin, TX, USA) (see **SI Text** for details). The samples were pooled at a concentration of 200 mM (quantified using quantitative real-time-PCR) into 6 different lanes for high-output 2 x 100bp paired-end sequencing. The bio-informatics analyses were performed on the Harvard FAS Odyssey Cluster (see **SI Text** for details). In brief, the reads were trimmed and then mapped to the *Gallus gallus* genome. The number of aligned reads for each sample was compiled and used for differential expression analysis, which was performed using DESeq2 (Love et al. 2014). For the associative pallium analyses, the model *[reads ~ region + species]* was run to obtain a measure of the associative pallium while taking into account variation cause by individual regions. For individual region analyses, a one-factor model *[reads ~ species]* was run. The principal component and hierarchical analyses were performed in DESeq as well. GO enrichment analysis was performed using the DAVID 6.8 functional annotation clustering tool (Huang et al. 2009). The network analysis was performed using the WGCNA package (44), using raw number of reads.

***In situ* hybridization.** Another cohort of 5 naïve birds per species was captured for *in situ* hybridization. Brain tissue hybridizations were performed as described in (Wada et al. 2006). In brief, ^{35}S-labeled riboprobes were made from T3, T7 or SP6 promoter sites of cDNA clones from (Wada et al. 2004) using T3, T7 or SP6 RNA polymerases (Roche). 10 μm fixed sections on slides were hybridized for 16h at 65°C. They were then washed, dehydrated and exposed in a autoradiography cassette with a Kodak BioMax MR film in the dark at 4°C for 48-72h before developing the films. Optical

densities were then measured with ImageJ2. To assess differences in individual regions (data presented in **Fig. 4A, Fig. S10A** and **Table S6**), we performed two-way ANOVAs using data from all regions and report interspecific Bonferroni post-test comparisons for each region. To test for differences in the associative pallium (data presented in **Fig. 3B**), we performed two-way ANOVAs and report differences for the species factor, and then applied Bonferroni corrections for multiple comparisons.

Immunohistochemistry. The same animals from *in situ* hybridization were used for immunohistochemistry. Fixed sections were blocked using Bloxall (# cat SP-6000, Vector Labs), rinsed and incubated in normal blocking serum. Sections were then incubated in 1:500 primary antibody (anti-GRIN2A, cat # Ab118587; anti-GRIN2B, cat # Ab65783, Abcam, Cambridge, UK) overnight at 4°C. They were then incubated with appropriate secondary antibodies followed by ABC reagent (cat # PK-6100, Vector Labs). Finally, they were washed and incubated in DAB peroxidase substrate (# cat SK-4100, Vector Labs) and coverslipped. They were quantified the same way as in the *in situ* hybridization analysis.

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Figures

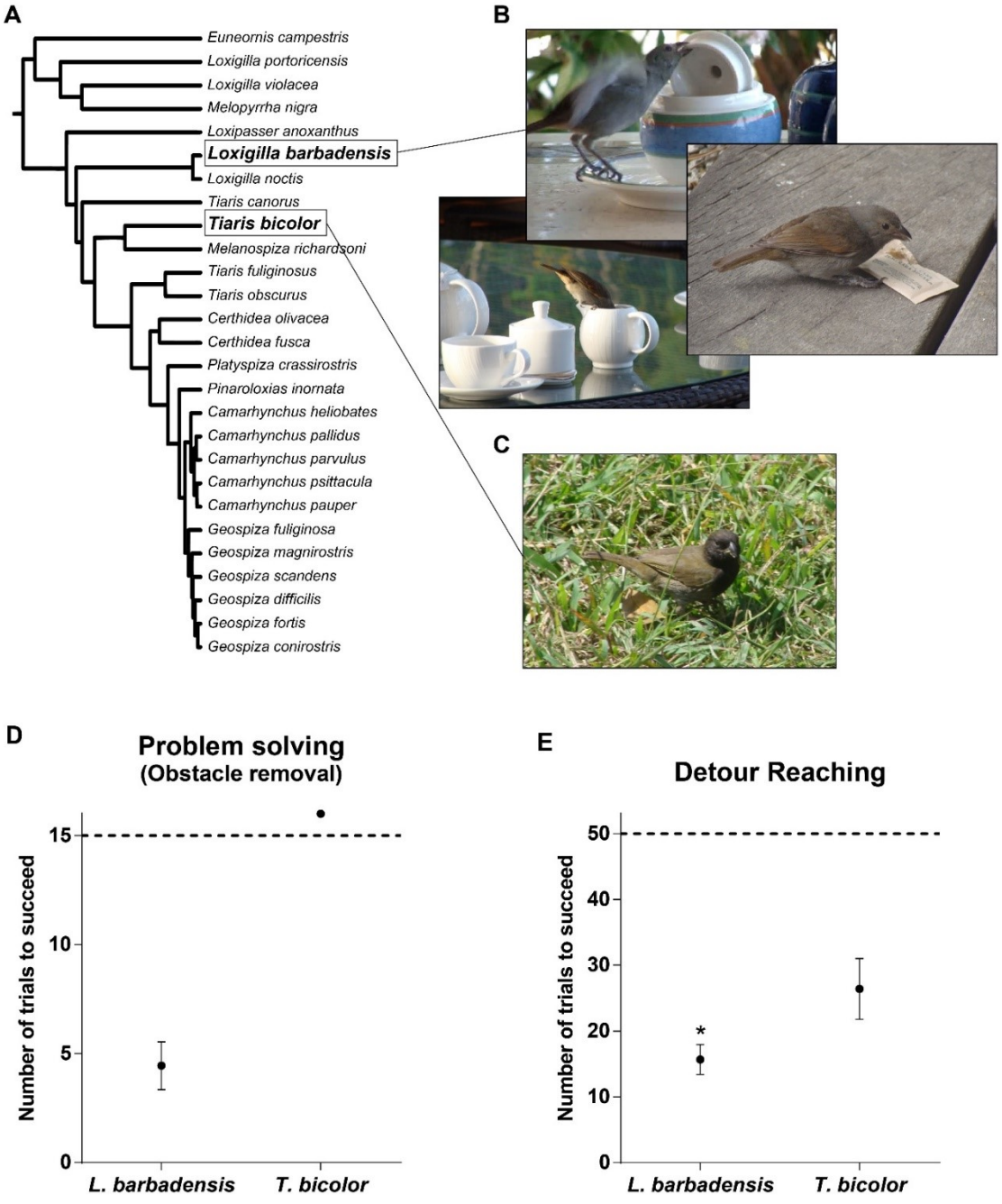

Figure 1. Study species. (**A**) Portion of the phylogenetic tree of the *Thraupidae* family that includes *Tiaris bicolor* and *Loxigilla barbadensis* (Barker et al. 2015). (**B**) In the wild, *L. barbadensis* is bold, opportunistic and highly innovative whereas (**C**) *T. bicolor* is shy, conservative and non-innovative. (**D**) Number of trials needed to complete the obstacle removal problem. *L. barbadensis* completed the task in a mean of 4.4 ± 1.09 trials, but none of the tested *T. bicolor* solved it within the 15 allocated trials. (**E**) The number of trials to reach the success criterion in the detour reaching task was lower in *L. barbadensis* (15.7 ± 2.3 trials, n=29) than in *T. bicolor* (26.4 ± 4.6 trials, n=15; * $P_{Mann\text{-}Whitney} = 0.0143$).

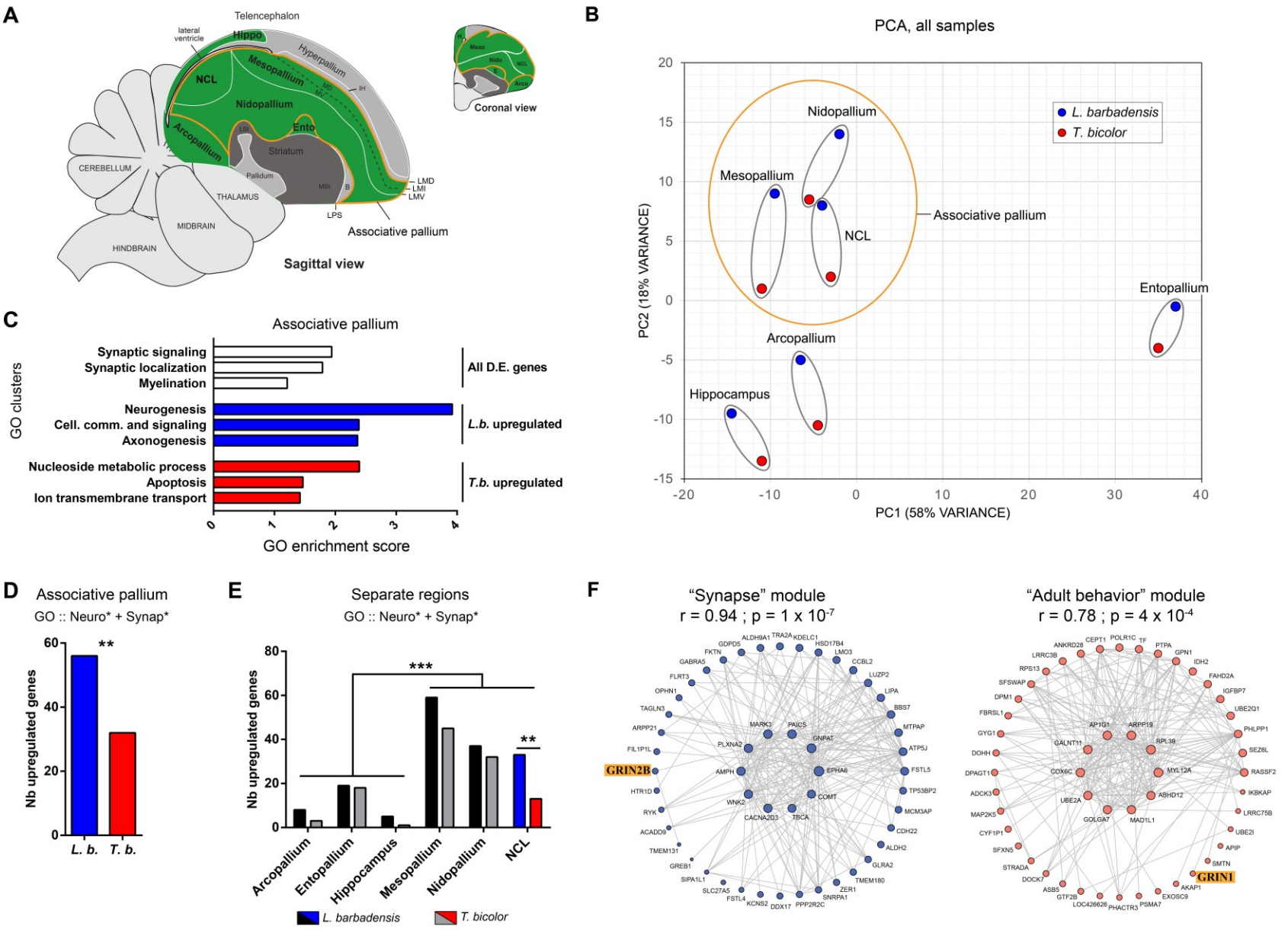
A
Telencephalon
lateral ventricle
Hippo
Hyperpallium
Mesopallium
NCL
Nidopallium
Ento
Arcopallium
Striatum
Pallidum
CEREBELLUM
THALAMUS
MIDBRAIN
HINDBRAIN
LMD
LMI
LMV
LPS
Associative pallium
Sagittal view
Coronal view
B
PCA, all samples
L. barbadensis
T. bicolor
Nidopallium
Mesopallium
NCL
Associative pallium
Entopallium
Arcopallium
Hippocampus
PC2 (18% VARIANCE)
PC1 (58% VARIANCE)
C
Associative pallium
Synaptic signaling
Synaptic localization
Myelination
Neurogenesis
Cell. comm. and signaling
Axonogenesis
Nucleoside metabolic process
Apoptosis
Ion transmembrane transport
All D.E. genes
L.b. upregulated
T.b. upregulated
GO clusters
GO enrichment score
D
Associative pallium
GO :: Neuro* + Synap*
Nb upregulated genes
L. b.
T. b.
E
Separate regions
GO :: Neuro* + Synap*
Nb upregulated genes
Arcopallium
Entopallium
Hippocampus
Mesopallium
Nidopallium
NCL
L. barbadensis
T. bicolor
F
"Synapse" module
r = 0.94 ; p = 1 x 10-7
GRIN2B
"Adult behavior" module
r = 0.78 ; p = 4 x 10-4
GRIN1

Figure 2. RNA-Seq analysis of *L. barbadensis* and *T. bicolor* transcriptomes. (**A**) Schematic view of the avian brain (Jarvis et al. 2013), with the regions that were examined in this study colored in green. The orange outline designates the regions that form the associative pallium. Hippo: Hippocampus; NCL: Nidopallium caudolaterale; IH: Intercalated hyperpallium; MD: Dorsal mesopallium; MV: Ventral mesopallium; Ento: Entopallium; LSt: Lateral striatum; Mst: Medial striatum; B: Basorostralis; LMD: Lamina mesopallium dorsale; LMI: Lamina mesopallium intermediate; LMV: Lamina mesopallium ventrale; LPS: Lamina pallio-subpallialis. (**B**) Principal component analysis of gene expression pattern per species and per region. Individual blue (*L. barbadensis*) and red (*T. bicolor*) circles include the mean of the reads from all individuals for a given species/region. (**C**) Gene ontology (GO) clustering analysis of differentially expressed genes, using, separately, the whole dataset of differentially expressed genes, the genes that are upregulated in *L. barbadensis* or the genes that are upregulated in *T. bicolor*. The three clusters with the highest enrichment scores are shown (all $P < 0.05$ except myelination $P = 0.0517$). For all GO terms for each cluster, see **Tables S2, S3 and S4**. (**D**) Considering only the genes that are characterized by synaptic and neuronal GO terms, the number of genes that are upregulated in *L. barbadensis* is higher than the number of genes that are upregulated in *T. bicolor* in the associative pallium. $^{**}p < 0.01$. (**E**) Using the same subset of genes, the number of genes that are upregulated in *L. barbadensis* compared to the number of genes that are upregulated in *T. bicolor* in each of the regions. *L. barbadensis* had more upregulated genes in the NCL. $^{**}p < 0.01$. The total number of differentially expressed genes is significantly higher in the associative pallium than in the three other regions. $^{***}p < 0.001$. (**F**) Two significant constructed network modules: "Synapse" and "Adult Behavior". See **Table S5** for all clustered GO terms per module and see **Figure S9** for all other modules. Both have a positive r value, indicating that the mean expression in the modules is higher in *L. barbadensis* compared to *T. bicolor*. Highlighted genes are glutamate receptors that were found to differ significantly following the differential expression analyses presented in **Figure 3**.

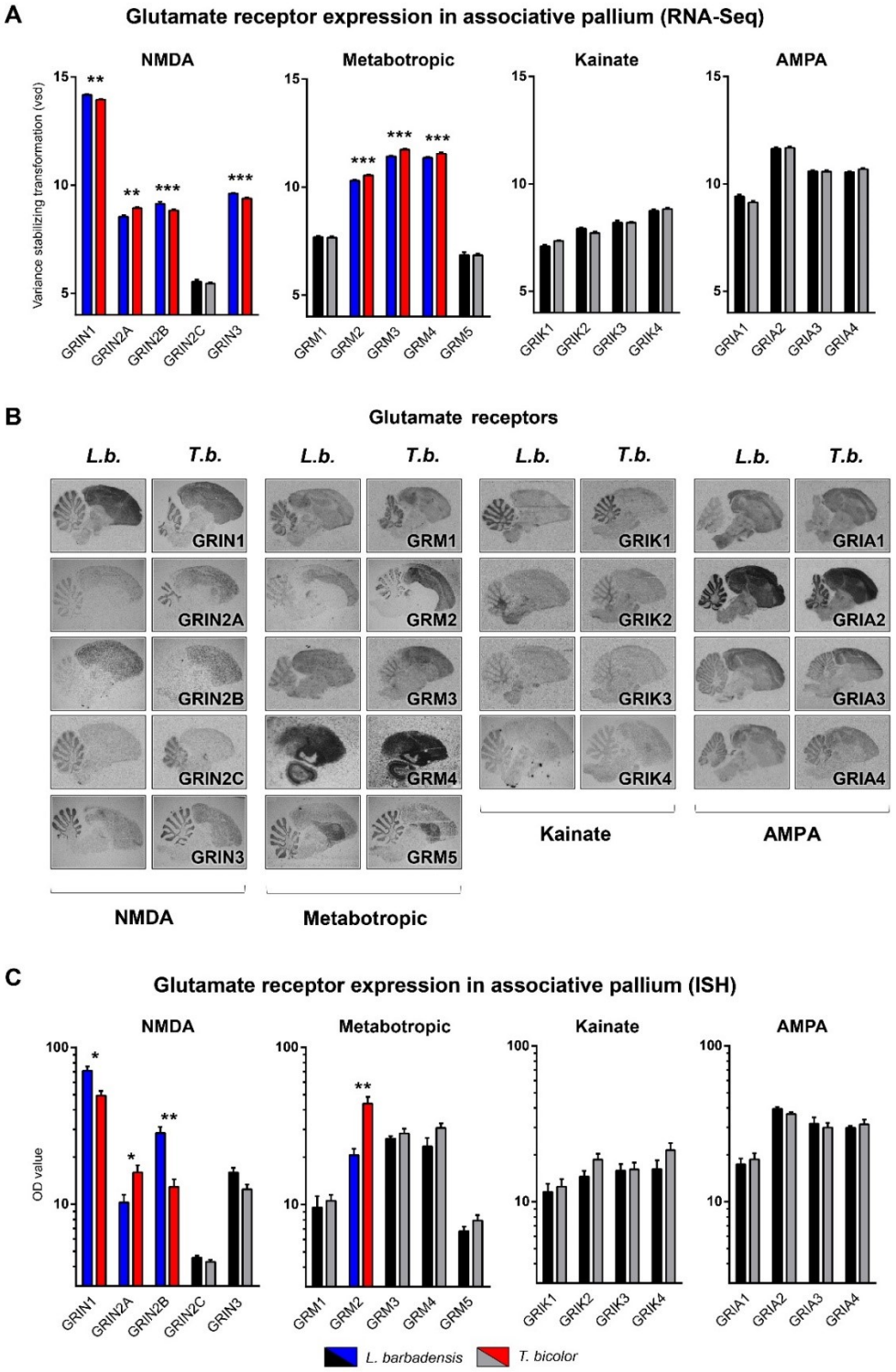

Figure 3. Glutamate receptor expression analysis. (**A**) RNA-Seq data (variance stabilizing transformation of reads) of all glutamate receptors. P-values were obtained by differential expression analysis. (**B**) Representative autoradiography images of glutamate receptor *in situ* hybridizations. (**C**) Quantification of the signal obtained by *in situ* hybridization for all assessed glutamate receptors. Significantly different expression is indicated by colored bars. *p < 0.05, **p < 0.01, ***p < 0.001.

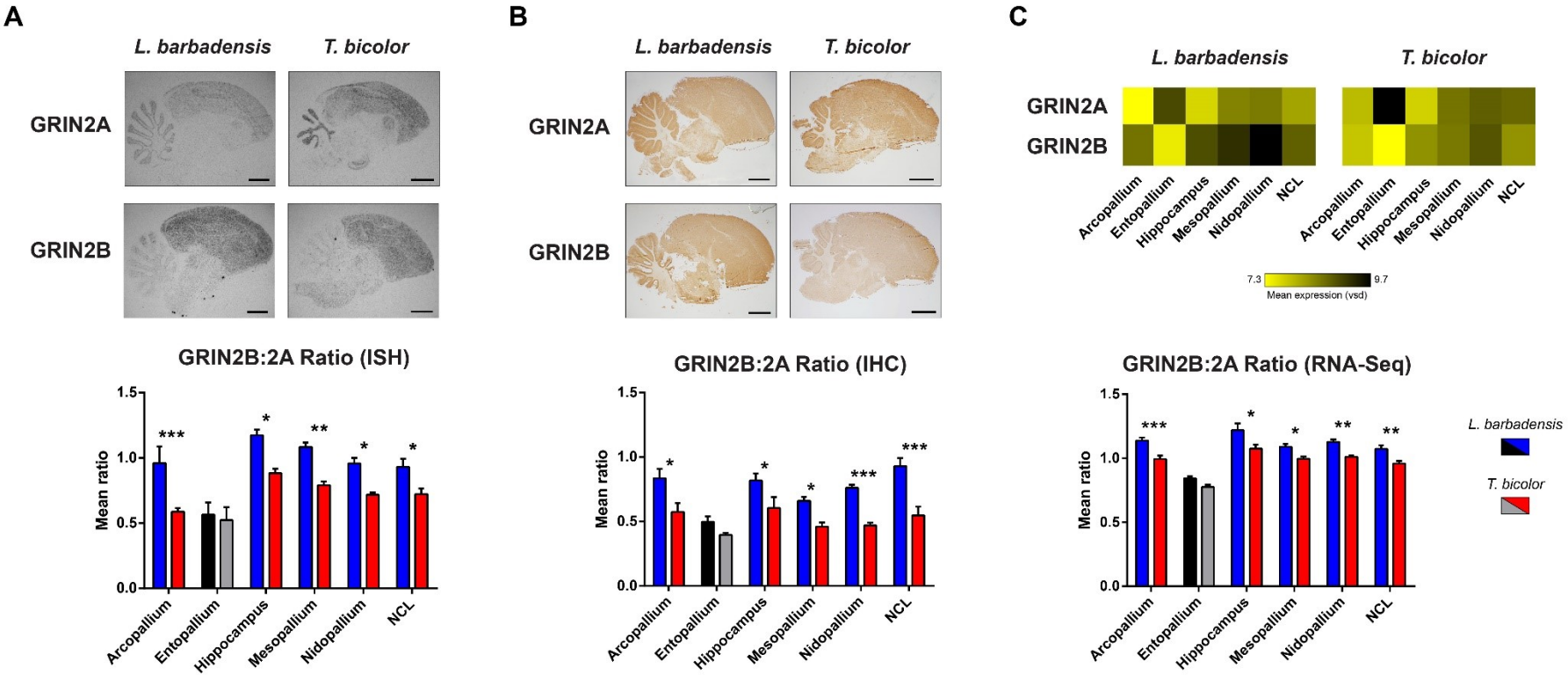

Figure 4. GRIN2A and GRIN2B expression across all brain regions. (**A**) *In situ* hybridizations of GRIN2A and GRIN2B mRNA with their mean GRIN2B:GRIN2A ratios below, calculated with the quantifications of individual receptor expression in each region. (**B**) Immunohistochemistry targeting proteins with GRIN2A- and GRIN2B-specific antibodies, with their mean GRIN2B:GRIN2A ratios below, calculated with the quantifications of individual receptor expression in each region. (**C**) Heatmaps of mean expression (vsd transformed reads) for GRIN2A and GRIN2B from RNA-Seq data. Below, mean ratios calculated with individual receptors. Means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bars: 500 µm.

Supporting information

Materials and Methods

I. Behaviour

Capture and captivity conditions

All birds were captured using mist nets between February and May in Holetown, St. James, Barbados. To keep the number of tested individuals to a minimum and because of the potential influence of sex on the behavioural variables we measured, we aimed at testing only males. Since *T. bicolor* has an obvious colour dimorphism (black head, see **Fig. 1C**), we were able to capture only males (n=15); since *L. barbadensis* has no evident colour dimorphism, we tested 29 individuals of both sexes, aiming for equal numbers of males and females. With subsequent molecular sexing with PCR performed on DNA extracted from blood (Audet et al. 2014), we identified 17 males and 12 females. As sex did not have an effect on any of the behavioural measurements (see Table S1), all 29 *L. barbadensis* were kept for behavioural analyses. Another cohort of birds was captured for neurobiological analyses (see Brain sample processing - Brain collection), this time consisting of only males of both species, to avoid introducing confusion by sex-specific brain differences. Male *L. barbadensis* were first identified based on morphometric measurements (see 29) and we later confirmed by PCR that all individuals were males.

Upon capture, birds were banded and morphological measurements (weight, tail length, wing length, metatarsal length, bill length and head length) were taken three times in succession on each bird and the mean value of the three measures used (Audet et al. 2014).

Birds were then housed in individual cages (H: 92 cm x W: 73.5 cm x L: 81 cm) visually but not acoustically isolated from each other in an indoor aviary. Birds were first habituated to their cages for a period of two days, during which they were not disturbed and fed *ad libitum* a finch seed mix in 60 mm Petri dishes. At the end of day 2, birds were food-deprived overnight to increase their motivation to participate in the tests on the next morning. Water was always supplied *ad libitum*. Since *T. bicolor* has a lower body weight than *L. barbadensis* (T.b.: 10.1 ± 2.6 g; L.b.: 17.5 ± 2.7 g), we carried out a shorter food deprivation period for *T. bicolor* (12h) than for *L. barbadensis* (14.5h) to obtain

comparable levels of motivation. For *T. bicolor*, behavioural tests were started at 6:30 and finished at 13:00, and for *L. barbadensis*, tests were started at 9:00 and finished at 15:00.

Temperament

On day 3, a boldness assessment was performed by presenting the feeding dish (the same as during the 2-day habituation period) and then measuring the latency to feed after the experimenter left (Audet et al. 2016). The experimenter hid behind a curtain and watched through a hole until the bird fed. Each trial lasted 20 min and a maximum score of 1201 s was given for birds that never fed during the boldness trial. The obstacle-removal problem-solving apparatus (a transparent cylinder of 3 cm x 3 cm filled with seeds, with a removable lid, **Fig. S1A**) was then presented for the first time with the lid opened and the latency to feed from it was used as the neophobia measurement, from which the boldness latency (see above) was subtracted. This provides a useful measurement of each bird's specific avoidance of the apparatus, which could potentially confound the problem-solving score.

Problem-solving

Once a bird had fed from the open apparatus, the first problem-solving trial began, with the lid loosely fitted and covering the top of the cylinder. The trials lasted for 5 min each with an inter-trial interval of 5 min. If a bird did not solve the task within the maximum of 15 trials, it was attributed a score of 16 trials for the initial problem-solving assessment. Then, all the unsuccessful birds were shaped, i.e. trained to solve the task. The subjects were presented with progressively more difficult levels of the task (**Fig. S2C**): level 0, where the cylinder was open and the lid was placed beside the apparatus; level 1, half of the opening of the cylinder was covered by the lid placed bottom-up; level 2, three-quarters of the opening was covered, lid bottom-up; level 3, the opening was fully covered, lid bottom-up; level 4, opening fully covered, lid bottom-down, as in the initial problem-solving assessment. Two consecutive successes at each level led to the presentation of the next level. Upon first failure at the harder level, the easier level was presented once again. The maximum number of shaping trials was set at 60 5-min trials. Unsuccessful individuals were given a score of 76 trials (60 shaping + 15 initial + 1). All successful birds were given the task again to confirm that they did not succeed by chance; all birds solved it in the first trial of this confirmation phase (mean latency: 83 ± 15 s).

Detour-reaching

The detour-reaching task consisted in an apparatus that prevented direct access to a visible food reward, requiring instead the birds to inhibit their first approach of pecking at the transparent barrier separating them from the food (Boogert et al. 2011). The birds were first trained using an opaque 1-inch PVC cylinder, at the centre of which two seeds were placed (**Fig. S1B**, left). To succeed in this phase, the subjects had to reach for the seed inside the cylinder without pecking at the outside. Once they had learned to directly reach for the seed for 7 trials in a row, they were presented with the testing apparatus, a transparent cylinder of identical dimensions as the previous opaque one (**Fig. S1b**, right). Again, the success criterion was to reach directly for the seeds, without pecking at the transparent cylinder, for 7 trials in a row.

Discrimination learning

The discrimination learning procedure followed the protocol described in Audet et al. (2016). In brief, the birds had to associate a given colour with a food reward (seeds in a Petri dish placed on a coloured platform; **Fig. S1C**). The incorrect colour had an identical Petri dish placed in it, but it had seeds glued to the bottom of the dish, thus yielding no reward. Both coloured platforms were first presented with available, non-glued, seeds as a "colour bias" trial. The chosen colour became the unrewarded one for the subsequent learning trials. Platforms were then presented with alternated left-right locations and removed either immediately when the bird had chosen the wrong colour or after it had eaten for 15 s if it had chosen the correct one. The learning criterion was choice of the correct colour on 7 consecutive trials. Reversal learning was assessed on the following day by switching the rewarded colour and measuring the number of trials to achieve the same success criterion as in the initial learning phase. On the first reversal learning trial, all birds chose the previously learned colour, indicating that they associated the colour with the reward rather than other cues (e.g. visual or olfactory cue of the rewarded dish versus the glued seeds dish).

Ethical statement

After behavioural experiments were completed, birds were released at their initial site of capture. All experiments were conducted according to Animal Use Protocol # 2012-7140 approved by the McGill University Animal Care Committee and permit # 8434/56/1 from the Natural Heritage Department of the Barbados Ministry of Environment, Water Resources Management and Drainage.

II. Brain sample processing

Brain collection

Brains were all collected during February in Holetown, St. James, Barbados. We captured a different cohort of naïve animals from the ones used in the behavioural experiments to prevent the modulation of brain mRNA caused by the stress of captivity and the experience of the behavioural tests. For the same reason, individuals were sacrificed as quickly as possible after capture, considering the fact that the birds were caught in the wild and that they needed to be brought back to the lab for appropriate euthanasia (time of death: 4.7 ± 0.2 min after capture).

The first cohort consisted in 8 captured male birds per species for RNA-Seq. The birds were decapitated and brains were excised. The brain hemispheres were put immediately in vials containing a RNAlater stabilization solution (cat # AM7021, ThermoFisher, Waltham, MA, USA) and kept on ice or at 4°C until shipping to the laboratory (which took less than a week; RNAlater solution allows for storage of RNA at 4°C for up to 4 weeks without significant degradation) and then stored at -20°C until mRNA extraction.

Another cohort of 5 naïve male birds per species was captured for *in situ* hybridization. The birds were decapitated, brains were excised, hemispheres separated, and put in a block mold containing Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA, USA), then immediately frozen on dry ice. The whole procedure lasted 4.4 ± 0.2 min. Blocks were kept on dry ice or in a -80°C freezer until sectioning.

III. RNA-Seq

Brain dissections

All brain dissections were performed in the lab of Erich Jarvis at Duke University. Brains were removed from RNAlater, quickly rinsed twice in PBS 1X with protease inhibitor cocktail (cat # 11697498001, Sigma-Aldrich, St. Louis, MO, USA) and rehydrated in the same solution for 5 min on ice. At this point, the two hemispheres were independently processed. The medial part of the hemisphere was mounted against a thin plastic wall with Vetbond tissue adhesive (3M, St. Paul, MN, USA), placed on the surface of a tissue slicer (cat # 51415, Stoelting Co., Wood Dale, IL, USA). 400 μm thick sagittal sections were cut, and the sections were placed in PBS with protease inhibitor in a Petri dish installed on a custom frozen stage. The Petri dish with the sections, on the frozen stage, was then placed under a dissecting microscope (Olympus MVX10), and regions of interest quickly dissected (within 5-15 min) with fine scissors and forceps (Fine Science Tools USA, Foster City, CA) and put in tubes containing RNAlater. Samples from both hemispheres were pooled, but samples from individual birds were kept in separate tubes, resulting in 8 biological replicates per species. Tubes were always kept at 4°C during procedure, and then at -20°C until further use.

Library preparation

The remaining experiments for library preparation and RNA-Sequencing were conducted in the lab of Lauren O'Connell at Harvard University. Total RNA was first extracted from brain samples using a standard Trizol (cat # 15596018, ThermoFisher, Waltham, MA USA) procedure. mRNA was then purified using the MicroPoly(A)Purist Kit (cat # AM1919, ThermoFisher, Waltham, MA USA). Briefly, this kit enriches for poly(A) RNAs from total RNA samples, by hybridizing the poly(A) sequences found on most mRNAs with Oligo(dT) cellulose, that is then transferred on a spin column and washed to remove nonspecifically bound material and ribosomal RNA. Libraries were then prepared using the NEXTflex™ Directional RNA-Seq Kit (dUTP-Based) (cat # 5129-06, Bioo Scientific, Austin, TX, USA). This kit prepares mRNA samples for Illumina sequencing using magnetic beads by: i) fragmenting mRNAs; ii) synthesizing the first DNA strand from mRNA using a reverse transcriptase; iii) synthesizing the second DNA strand; iv) repairing

the strand ends using enzymes; v) adenylating fragments; vi) ligating different adapters (24 unique adapters allowing multiplexing of the samples – up to 24 per sequencing lane) to fragments of each sample individually; and vii) amplifying DNA by PCR. The samples were then pooled at a concentration of 200 mM (quantified using quantitative real-time-PCR) into 6 different lanes for sequencing.

Sequencing

Samples were sequenced at the FAS Center for Systems Biology at Harvard University (Cambridge, MA, USA) in an Illumina HiSeq 2000 sequencer, using a high-output 2 x 100bp paired-end sequencing protocol. We distributed all samples in 6 lanes: each lane had one region with all individuals (8 biological replicates per species = 16 samples per lane).

Data pre-processing

All the following manipulations and analyses were performed on the Harvard FAS Odyssey Cluster, on CentOS release 6.5 running Linux 2.6.32. The reads were first "trimmed" to remove the adapter sequences using Trimmomatic 0.30 (Bolger et al. 2014). Quality check was performed using FastQC 0.10.1 (Babraham Bioinformatics, Cambridge, UK).

Read Mapping

Reads were mapped to the Ensembl *Gallus gallus* genome (Aken et al. 2016) with the Burrow-Wheeler Aligner (BWA) tool, using the BWA-mem algorithm (Li 2013 Mar 16). Obtained SAM (Sequence Alignment/Map) alignment files were then sorted and converted to the BAM (compressed SAM) format using SAMtools (Li et al. 2009). HTSeq-count tool (Anders et al. 2015) was used to obtain the number of aligned reads for each feature (Ensembl IDs). Gene names were obtained from the Ensembl ID numbers using the Ensembl Biomart tool (Aken et al. 2016).

Differential expression (DE) analysis

DE analysis was conducted using DESeq2 in R (Love et al. 2014). The tables generated by HTSeq (raw reads) were imported in R, and the genes that had zero reads mapped to them for all samples were removed prior to performing DE analysis.

To obtain interspecific differences in the associative pallium, we used all samples of mesopallium, nidopallium and NCL. Analysis was run using the following model in DESeq2: *[reads ~ region + species]*, which yields a species' fold change value for every gene, taking into account the variation caused by regions. A total of 48 samples were thus analysed (3 regions x 2 species x 8 individuals).

To assess interspecific differences in gene expression for each region (data presented in Table S6), we performed DESeq2 analysis using each region's read counts independently in a one-factor model: *[reads ~ species]*. 16 samples were run per analysis (1 region x 2 species x 8 individuals), and it was repeated for each region. MA plots of log2changes dispersion (Figure S8) were generated using the "plotMA" function in DESeq2 using DESeqDataSet for each region.

For all DE analyses, we used DESeq2-adjusted P-values.

Interspecific differences in number of DE genes

To assess differences in the number of upregulated genes in *L. barbadensis* compared to *T. bicolor* in the six regions, we ran chi-square tests with the number of differentially expressed genes in each species compared to a 50% distribution of the same DE genes. Analysis was performed using significantly differentially expressed ($p < 0.05$) genes of a known neurological role (with gene ontology terms containing "neuro" or "synap"), with high expression (reads > 50), and high difference in gene expression between species (log2 fold change > 0.5).

Principal component analysis

To perform the principal component analysis (**Fig. 2a**), we used the mean of the number of non-zero reads for all samples (n=8), for each region of each species. The analysis was performed using the "plotPCA" function from DESeq2 with variance stabilizing transformed values (Love et al. 2014).

Hierarchical cluster analysis

To perform the hierarchical cluster analysis (**Fig. S5**), we used averages of all non-zero reads from all samples of corresponding regions of both species. The heatmap with related

distances tree were constructed using the "distsRL" function of DESeq2 with variance stabilizing transformed values (Love et al. 2014).

Gene ontology term enrichment analysis

GO enrichment analysis was performed using the DAVID 6.8 functional annotation clustering tool (Huang et al. 2009). Parameters were adjusted according to the number of genes included per analysis: for all DE genes, we used the "highest stringency" option; for DE genes per species, we used the "high stringency" option; for the individual modules, we used the "medium stringency" option. We kept clusters in which all member had >10 counts. All differentially expressed genes with a minimum expression of 50 reads were included in the analyses. We used all biological processes of GO categories as well as functional categories defaults.

Network analysis

Network analysis was performed using the WGCNA package (Langfelder and Horvath 2008), using raw data (number of reads) from each individual, region and species (n=96 samples). Based on the Scale-free topology fit index generated by our data, we chose a soft-thresholding power of 10. An unsigned network was constructed using the following parameters: "bicor", minimum module size = 10, deep Split = 2 and dthresh = 0.1. The reported modules contain the 50 highest ranked genes, i.e. those with the highest kME.

IV. In situ hybridization

Brain sectioning and fixation

In situ hybridization experiments were performed in the lab of Erich Jarvis at Duke University. Brains were sectioned sagitally at a 10 µm thickness on a Leica cryostat and sections were placed directly on SuperFrost microscope slides (ThermoFisher, Waltham, MA, USA), dried and placed in a -80°C freezer until further use. On the day of *in situ* hybridization, the slides were taken out of the -80°C freezer and immediately fixed in 3% paraformaldehyde, rinsed, acetylated, dehydrated in graded (70%, 95%, 100%) alcohols and air-dried. They were then immediately hybridized.

Hybridization

Brain tissue hybridizations were performed as described in Wada et al. (2006). In brief, ^{35}S-labeled riboprobes were made from T3, T7 or SP6 promoter sites of cDNA clones from (Wada et al. 2004) using T3, T7 or SP6 RNA polymerases (Roche). Fixed brain sections were hybridized using 125 µL of hybridization solution per slide containing 1 x 10^6 cpm of ^{35}S-labeled riboprobe, 50% formamide, 0.3 M NaCl, 10 mM Tris-HCl pH 8.0, 12 mM EDTA pH 8.0, 1X Denhart's buffer, 0.01 M DTT, 0.5 mg/mL yeast tRNA, 0.25 mg/mL polyA and 0.2 mM Sodium dextran sulfate, coverslipped and immersed in a mineral oil bath for 16h at 65°C. The slides were then rinsed in chloroform and 2X SSPE + 0.1% β-mercaptoethanol following removal of coverslips. Subsequently, they were washed in new 2X SSPE + 0.1 % β-Me at RT°C for 1 hour, 2X SSPE + 50 % formamide + 0.1 % β-Me at 65°C for 1 hour and twice in 0.1X SSPE at 65°C for 30 minutes each. Slides were then dehydrated in consecutive graded (70%, 95% and 100%) alcohols and air-dried. The slides were put in an autoradiography cassette with a Kodak BioMax MR film in the dark at 4°C for 48-72h before developing the films.

Quantification

Digital images of autoradiography films were taken with an Olympus DP71 camera mounted on an Olympus MVX10 microscope. Images were then imported in ImageJ2 and optical density values for each separate region were measured. These values were normalized against background values. To assess differences in individual regions (data presented in **Fig. 4A, Fig. S10A** and **Table S6**), we performed two-way ANOVAs using data from all regions and report interspecific Bonferroni post-test comparisons for each region. To test for differences in the associative pallium (data presented in **Fig. 3B**), we performed two-way ANOVAs and report differences for the species factor, and then applied Bonferroni corrections for multiple comparisons.

V. Immunohistochemistry

Brain sectioning and fixation

The brains were sectioned using the same protocol as for the *in situ* hybridization. On the day of immunohistochemistry, the slides were taken out of the -80°C freezer, immediately fixed in 3% paraformaldehyde and rinsed in PBS.

Immunohistochemistry

We used the Vectastain Elite ABC kit (cat # PK-6100) from Vector Laboratories (Burlingame, CA, USA). The sections were first treated using Bloxall blocking Solution (# cat SP-6000, Vector Labs) for 10 min, rinsed in PBS-Tween and incubated in normal blocking serum (for GRIN2A: horse serum; for GRIN2B: goat serum) for 20 min. Sections were then incubated in 1:500 primary antibody (anti-GRIN2A, cat # Ab118587; anti-GRIN2B, cat # Ab65783, Abcam, Cambridge, UK) overnight at 4°C. Sections were rinsed twice in PBS-T, incubated in 1:200 biotinylated secondary antibody (for GRIN2A: anti-goat; for GRIN2B:anti-rabbit) for 30 min and washed twice in PBS-T. They were then incubated with the ABC reagent for 30 min and washed twice 5 min in PBS-T. Finally, sections were incubated with the DAB peroxidase substrate (# cat SK-4100, Vector Labs) for exactly 2 min each section and rinsed in distilled water. They were then dehydrated in graded alcohols, treated with xylenes and coverslipped.

Quantification

Digital images of immunohistochemistry slides were taken with an Olympus DP71 camera mounted on an Olympus MVX10 microscope. The intensity of the signal was quantified using the same protocol as for in situ hybridization quantification, and the same analyses were performed (data presented in **Fig. 4B** and **Fig. S10B**).

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Supplementary Figures and Tables

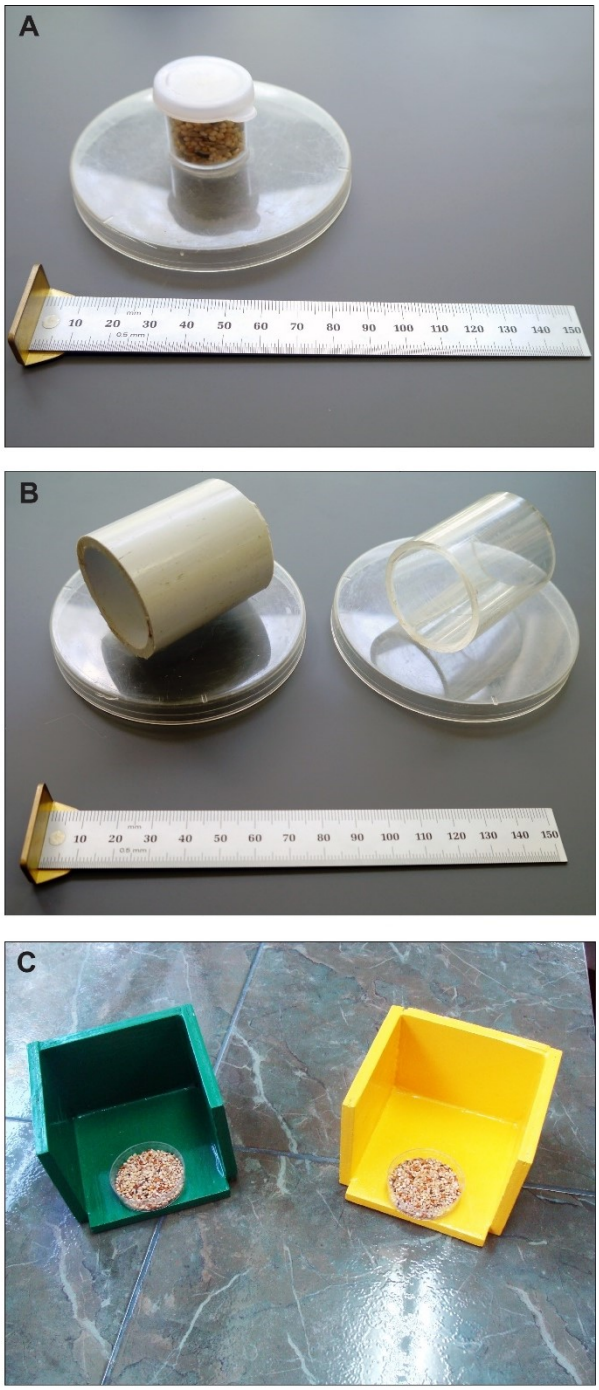

Figure S1. Behavioral tasks. (**A**) Obstacle removal problem. (**B**) Detour reaching task. The apparatus on the left was used for the training phase and the apparatus on the right used for the testing phase. (**C**) Discrimination learning apparatus. The seeds in one of the dishes cannot be eaten (they are glued to the bottom of the dish), making this color the non-rewarded one, although it looks identical to the other dish in which the seeds are available.

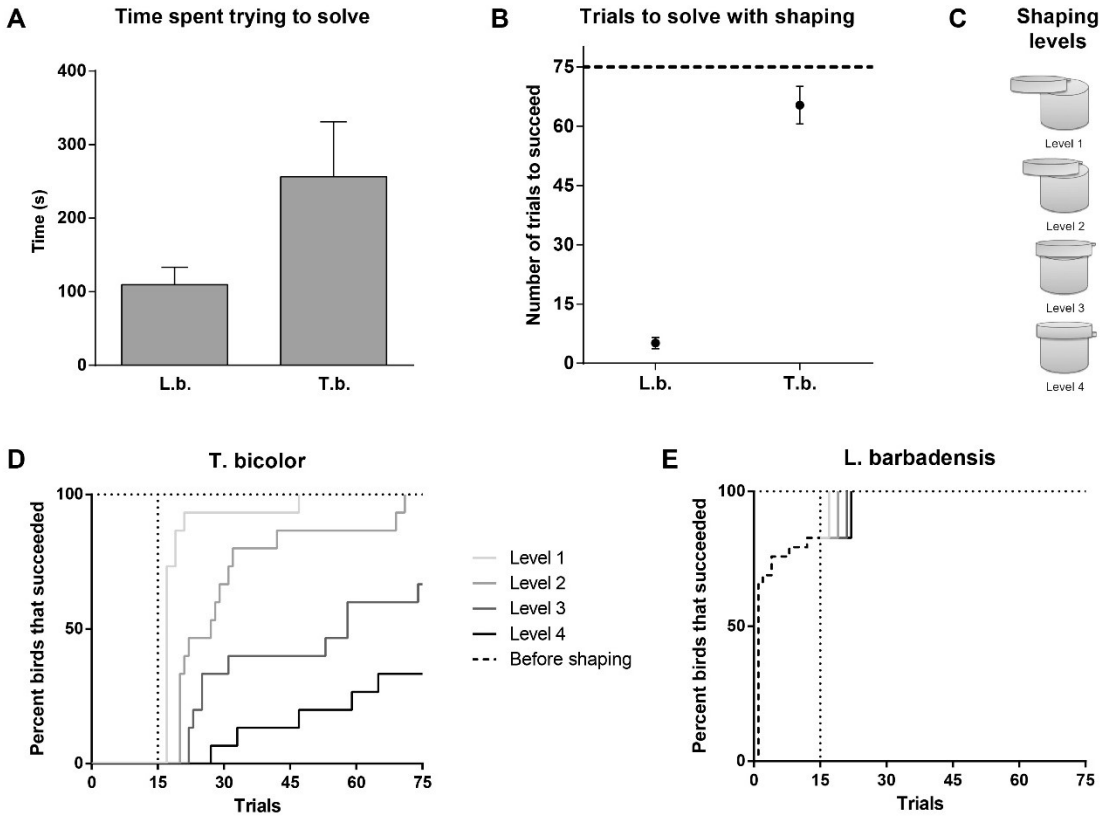

Figure S2. Problem-solving and shaping. (**A**) On average, the time spent trying to solve the obstacle removal task did not differ between the two species. (**B**) The total number of trials needed to solve the obstacle removal problem, including shaping trials, was significantly lower in *L. barbadensis* than in *T. bicolor*. (**C**) The shaping procedure consisted in gradually presenting four different levels of lid coverage in the obstacle removal task (see text for details). (**D**) Cumulative number of *T. bicolor* that solved the different levels of the task. None of the 15 individuals solved the task with a closed lid during the 15 trials before shaping (see Figure 2C). In the shaping phase, 100% of *T. bicolor* completed both levels 1 and 2, 66.7% level 3 and 33.3% level 4, before the maximum number of 75 trials was reached. (**E**) Cumulative number of *L. barbadensis* that solved the different levels of the task. 24 of 29 individuals solved the task with the closed lid during the 15 trials before shaping (see Figure 2C). The other 5 birds successively solved all shaping levels in the minimum possible number of trials. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, N.S.: non-significant.

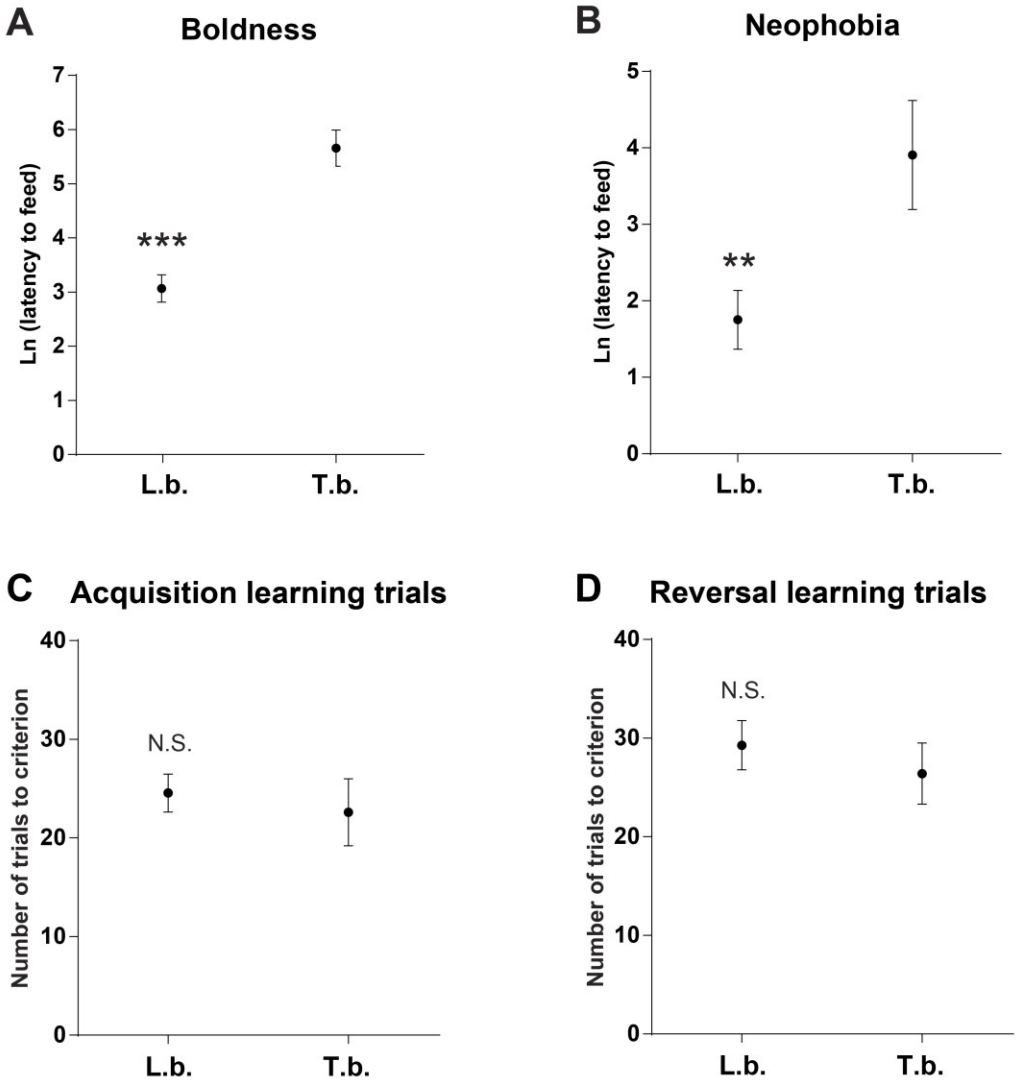

Figure S3. Risk-taking behavior. (**A**) Boldness, measured by the latency to feed following presentation of the dish by the experimenter, was lower in *L. barbadensis* (L.b.: ln latency to feed = 3.09 ± 0.25, n=30) compared to *T. bicolor* (*T.b.*: ln latency to feed = 5.66 ± 0.33, n=15; $P_{t\text{-}test} < 0.0001$). (**B**) Likewise, neophobia, measured as the latency to feed in the presence of a novel object (the problem-solving apparatus, see **SI Figure 1**), was lower in *L. barbadensis* (L.b.: ln latency to feed = 1.75 ± 0.39, n=29) than in *T. bicolor* (T.b.: ln latency to feed = 3.91 ± 0.71, n=15; $P_{t\text{-}test} = 0.0057$). (**C**) In the discrimination learning task, the number of trials to initially associate the correct color with the reward (see text for details) did not differ between species (L.b.: 24.6 ± 1.9 trials, n=29; T.b.: 22.6 ± 3.4 trials, n=15; $P_{Mann\text{-}Whitney} = 0.2814$). (**D**) Similarly, when the rewarded color was reversed with the previously unrewarded color, the two species did not differ in the number of trials to reach the success criterion (L.b.: 29.3 ± 2.5 trials, n=29; T.b.: 26.4 ± 3.1 trials, n=15; $P_{Mann\text{-}Whitney} = 0.4806$).

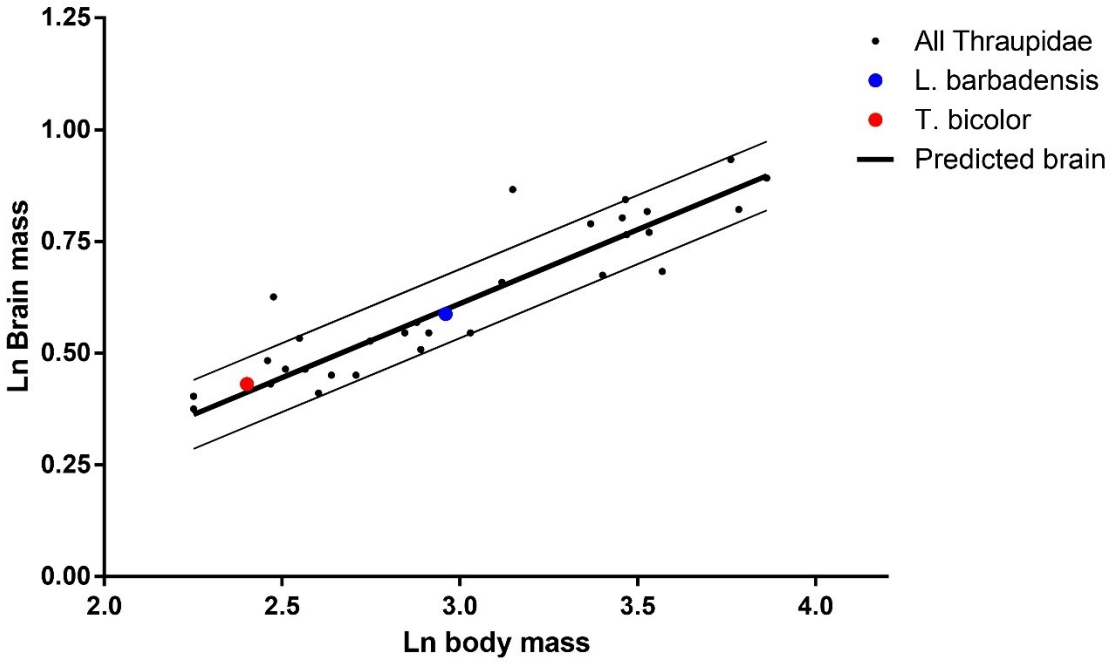

Figure S4. Residual brain masses of our two species vs other Thraupids. Regression (bold line) of PGLS-corrected brain mass against body mass for Thraupids (black dots), with the 95% confidence interval (fine lines). The residual values of *L. barbadensis* (blue dot) and *T. bicolor* (red dot) are within the confidence interval.

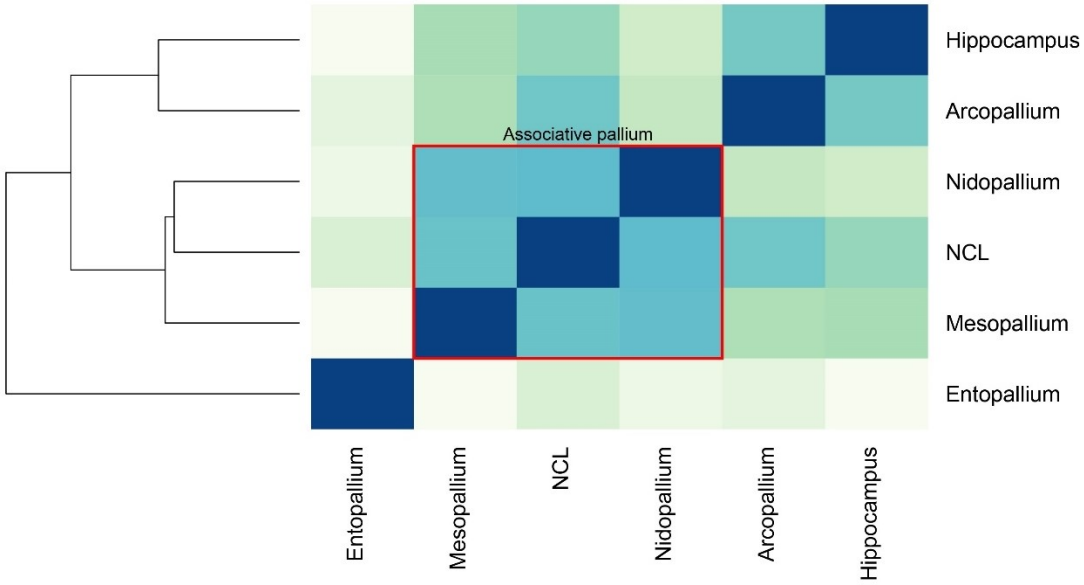

Figure S5. Cluster analysis of gene expression per region. Heatmap and the corresponding dendrogram based on the expression of all genes (variance stabilizing transformation of reads) in different regions. The three regions that form the associative pallium are marked by a red box.

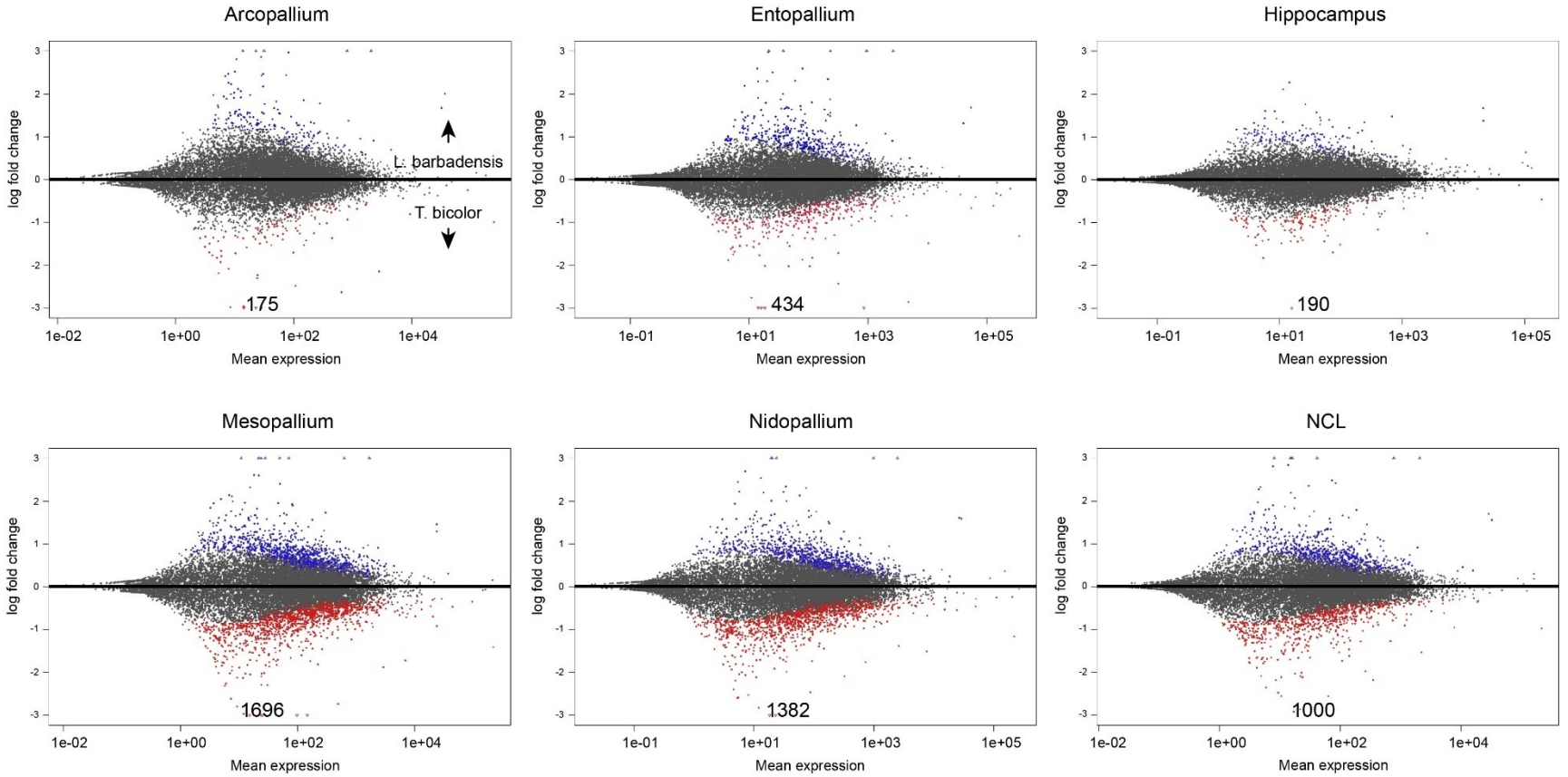

Figure S6. MA plots showing the distribution in the two species of fold change in all genes as a function of expression, for each analyzed brain region. Genes that are upregulated (i.e. more expressed) in *L. barbadensis* are plotted above the horizontal line while the ones that are upregulated in *T. bicolor* are plotted below the horizontal line. Significantly differentially expressed (DE) genes (adjusted p-value < 0.05) are plotted in blue (L.b.) and red (T.b.), and the total number of DE genes per region are indicated in the lower part of the graph.

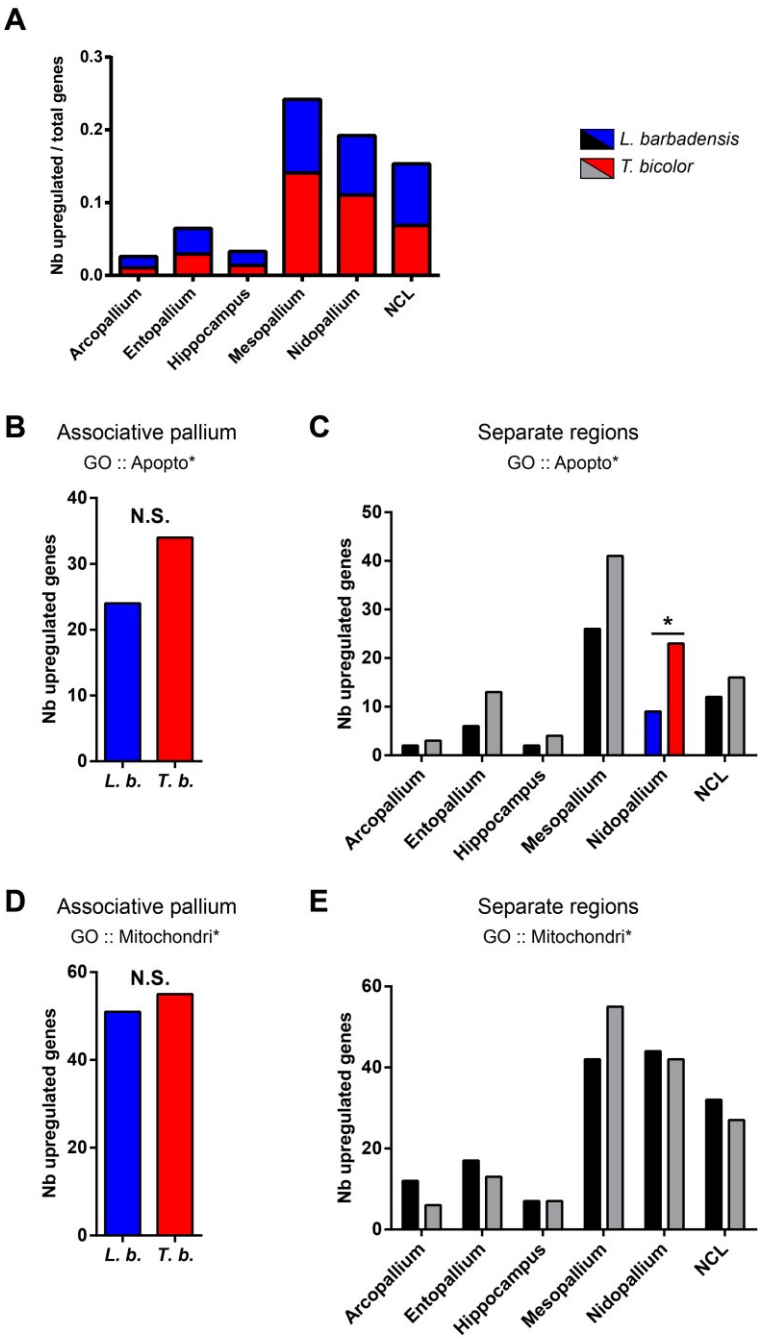

Figure S7. Proportion of differentially expressed genes and absolute number of upregulated genes for specific GO terms. (A**)** Bars show the total number of differentially expressed genes divided by the total number of expressed genes in this region, and colored portions show the proportion of genes that are upregulated in each species. (B) Considering only the genes that are characterized by apoptosis in their GO terms, the number of genes that are upregulated in either species is not significantly different in the associative pallium. (C) Using the same subset of genes, *T. bicolor* had more upregulated genes in the NCL than *L. barbadensis*. *$p < 0.05$. (D) There is no difference between the two species in the number of upregulated genes related to mitochondrion in the associative pallium. (E) Similarly, we did not find differences for the same genes in any of the individual regions.

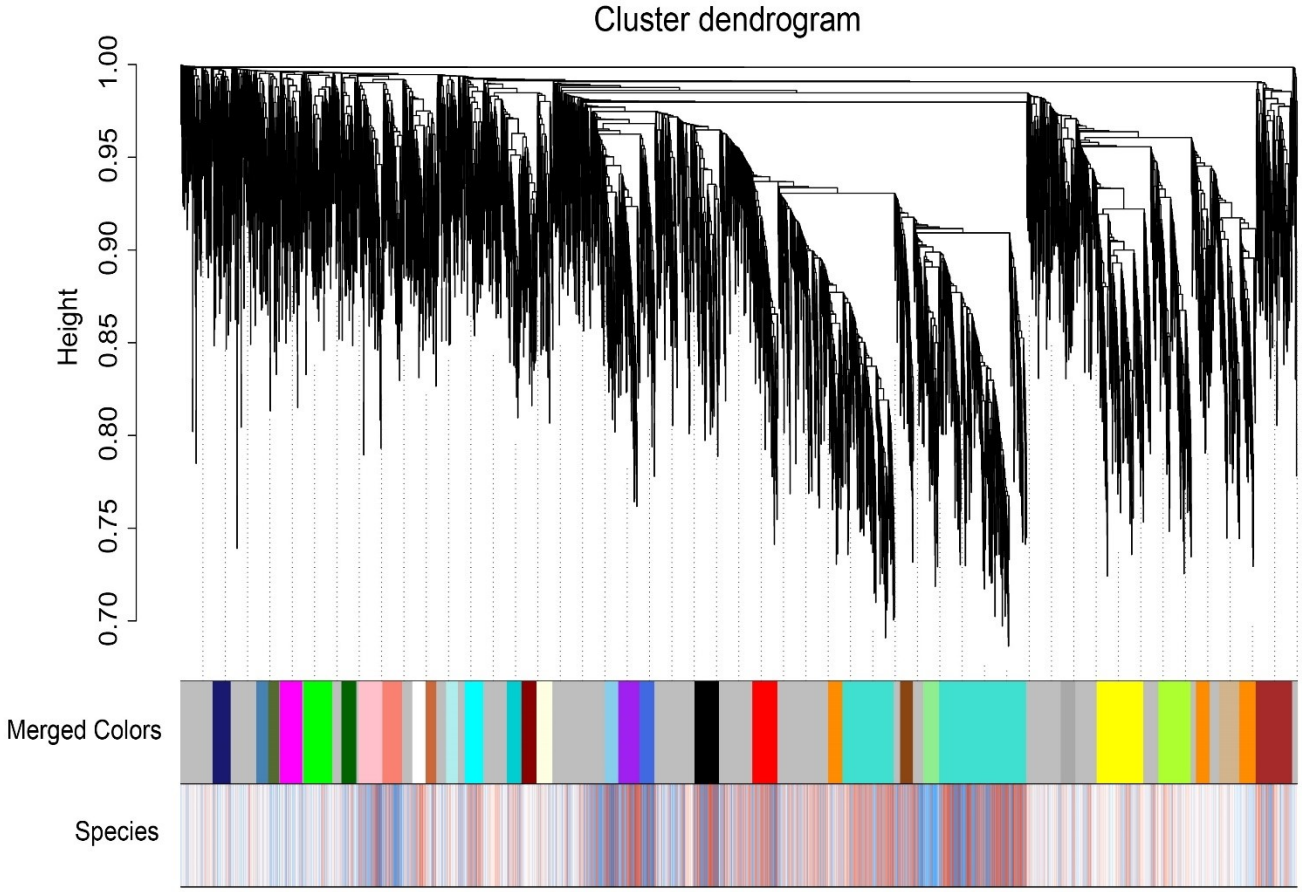

Figure S8. Network dendrogram. Clustering dendrogram of genes following weighted correlation network analysis of associative pallium gene expression, and below, assigned module colors and relationship with species (blue: higher in *L. barbadensis*, red: higher in *T. bicolor*, darker colors indicate higher expression).

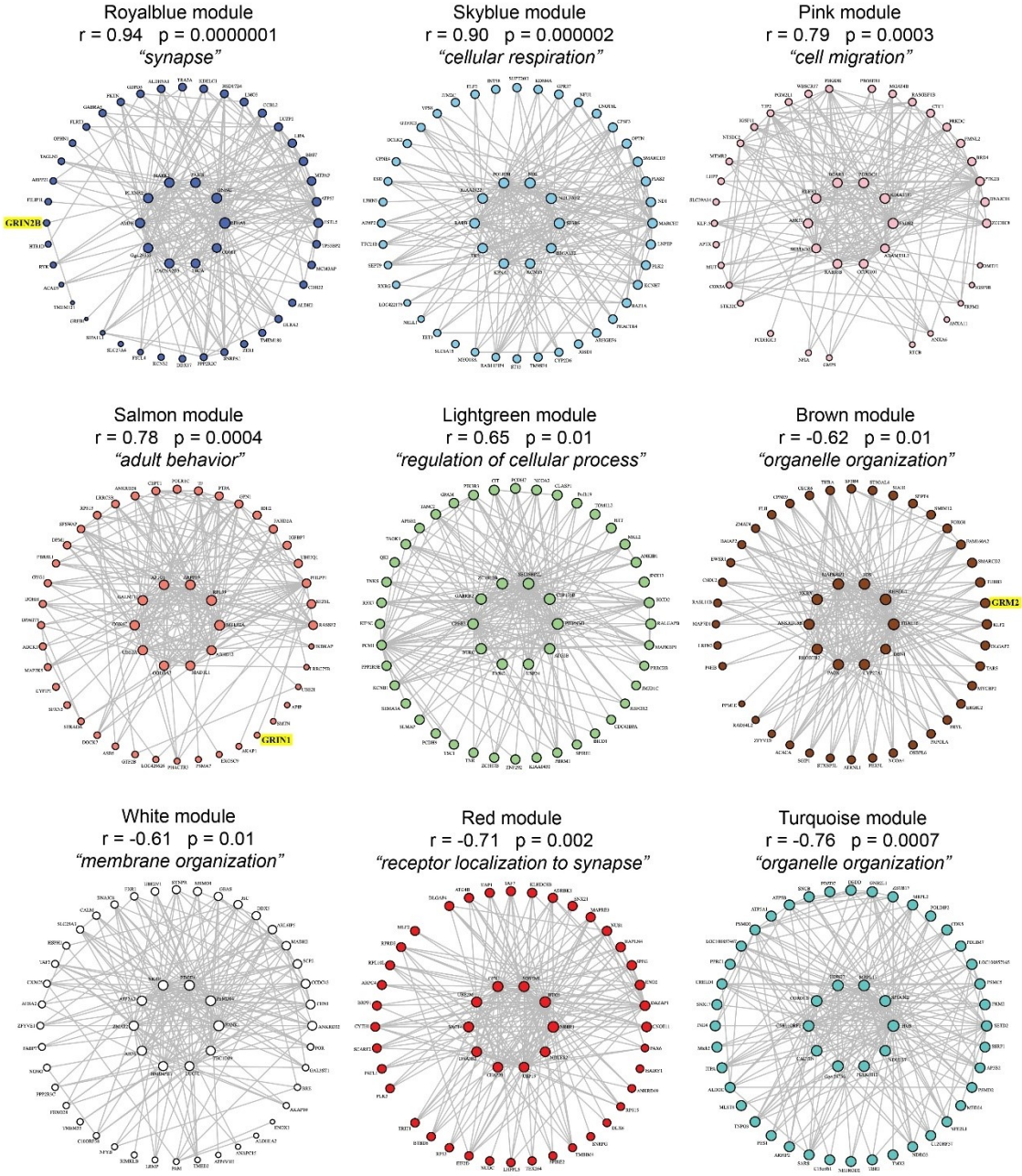

Figure S9. All modules following weighted correlation network analysis. All significant constructed network modules. Positive r values indicate networks with a higher mean expression in *L. barbadensis* and negative r values, a higher mean expression in *T. bicolor*. Highlighted genes are neurotransmitter receptors that were found to differ significantly following the differential expression analyses presented in **Figure 4**: GRIN2B in the Royalblue module, GRIN1 in the Salmon module and GRM2 in the Brown module. The gene ontology term that best defines the module based on functional clustering analysis is given in italics (See **SI Table 5** for all GO terms per cluster, for each module).

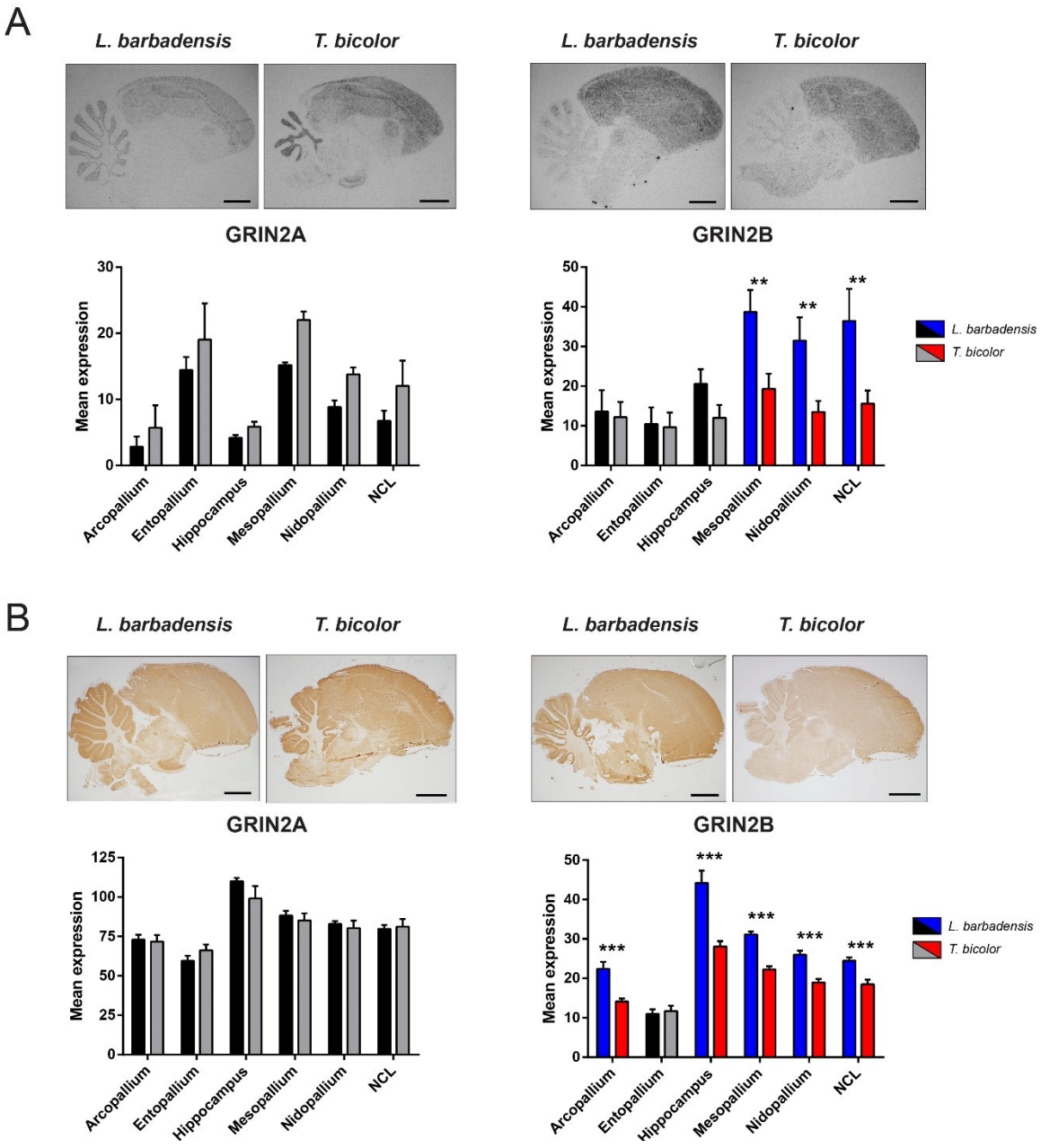

Figure S10. GRIN2A and GRIN2B expression across all brain regions. (**A**) *In situ* hybridizations of GRIN2A (left) and GRIN2B (right) with their quantifications below. *T. bicolor* expresses more GRIN2A overall (see **Figure 4B** and **C**), but when comparing each brain region, the differences between the species are not significant. However, the expression of GRIN2B was significantly higher in the mesopallium and nidopallium, including the caudolateral part of the latter (NCL), in *L. barbadensis*. (**B**) Immunohistochemistry with GRIN2A- and GRIN2B-specific antibodies, with their quantifications below. Again, the expression of GRIN2B was significantly higher in *L. barbadensis* in all regions except the entopallium.

Table S1. Linear model outputs for all behavioural variables. Significant explanatory variables for each behavioural trait measured, following stepwise variable selection of potential confounding variables that were included in initial models. The sign of the t value indicates in which direction the trait varies (the target variable is indicated in parentheses). Bold p-values $p < 0.05$.

Final model	r^2	**β**	***t***	***p***
*Problem solving (success/failure)**	n/a			
Species (L.b.)		**-10.93**	**35.53**	**< 0.0001**
Problem solving w/ shaping (trials)	0.88			
Species (L.b.)		**-26.45**	**-9.47**	**< 0.0001**
Boldness (latency)		-0.02	-2.68	0.0108
Detour reaching (trials)	0.12			
Species (L.b.)		**-5.43**	**-2.34**	**0.0242**
Boldness (latency)	0.45			
Species (L.b.)		**191.08**	**5.89**	**< 0.0001**
Neophobia (latency)	0.18			
Species (L.b.)		**-120.76**	**-3.09**	**0.0036**
Acquisition Learning (errors)	0.18			
Species (L.b.)		**2.85**	**3.05**	**0.0040**
Reversal Learning (errors)	0.10			
Acquisition Learning (errors)		0.48	2.18	0.0351
Reversal Learning (trials)	0.17			
Acquisition Learning (trials)		0.64	2.95	0.0052

*For problem-solving, we performed a GLMM with a binomial distribution. The chi-square is given instead of *t*.

Table S2. Gene ontology functional enrichment analysis for all differentially expressed genes in associative pallium. All terms per cluster that were significant, had n > 10 and an enrichment score > 0.1. Bold p-values p < 0.05.

Enrichment Score: 1.9359939620199742

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0098916~anterograde trans-synaptic signalling	54	2.90	**0.0116**	283	1.38
GO:0007268~chemical synaptic transmission	54	2.90	**0.0116**	283	1.38
GO:0099536~synaptic signalling	54	2.90	**0.0116**	283	1.38
GO:0099537~trans-synaptic signalling	54	2.90	**0.0116**	283	1.38

Enrichment Score: 1.78707018953527

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0048489~synaptic vesicle transport	17	0.91	**0.0136**	65	1.89
GO:0097480~establishment of synaptic vesicle localization	17	0.91	**0.0136**	65	1.89
GO:0097479~synaptic vesicle localization	17	0.91	**0.0236**	69	1.78

Enrichment Score: 1.2115548787715635

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0042552~myelination	12	0.64	0.0517	47	1.85
GO:0007272~ensheathment of neurons	12	0.64	0.0670	49	1.77
GO:0008366~axon ensheathment	12	0.64	0.0670	49	1.77

Enrichment Score: 0.7635931614824529

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0042455~ribonucleoside biosynthetic process	16	0.86	0.1647	83	1.40
GO:0009163~nucleoside biosynthetic process	16	0.86	0.1647	83	1.40
GO:1901659~glycosyl compound biosynthetic process	16	0.86	0.1887	85	1.36

Enrichment Score: 0.5892253090290928

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0006906~vesicle fusion	14	0.75	0.1613	70	1.45
GO:0090174~organelle membrane fusion	14	0.75	0.2010	73	1.39
GO:0044801~single-organism membrane fusion	14	0.75	0.5267	93	1.09

Enrichment Score: 0.5205701147967453

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030641~regulation of cellular pH	10	0.54	0.2661	51	1.42
GO:0051453~regulation of intracellular pH	10	0.54	0.2661	51	1.42
GO:0006885~regulation of pH	10	0.54	0.3876	57	1.27

Enrichment Score: 0.4550942815010804

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030335~positive regulation of cell migration	36	1.93	0.2924	230	1.13
GO:2000147~positive regulation of cell motility	36	1.93	0.3307	234	1.11
GO:0051272~positive regulation of cellular component movement	36	1.93	0.3904	240	1.09
GO:0040017~positive regulation of locomotion	36	1.93	0.4006	241	1.08

Enrichment Score: 0.4400498184138716

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0018393~internal peptidyl-lysine acetylation	14	0.75	0.3239	81	1.25
GO:0016573~histone acetylation	14	0.75	0.3239	81	1.25
GO:0006475~internal protein amino acid acetylation	14	0.75	0.3742	84	1.21
GO:0018394~peptidyl-lysine acetylation	14	0.75	0.4424	88	1.15

Enrichment Score: 0.39281942115670365

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0010631~epithelial cell migration	23	1.23	0.3634	146	1.14
GO:0090132~epithelium migration	23	1.23	0.4020	149	1.12
GO:0090130~tissue migration	23	1.23	0.4539	153	1.09

Enrichment Score: 0.3497510336479343

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030072~peptide hormone secretion	19	1.02	0.4052	121	1.14
GO:0002790~peptide secretion	19	1.02	0.4484	124	1.11
GO:0015833~peptide transport	19	1.02	0.4914	127	1.08

Enrichment Score: 0.2921464928109328

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0099643~signal release from synapse	10	0.54	0.4911	62	1.17
GO:0007269~neurotransmitter secretion	10	0.54	0.4911	62	1.17
GO:0099531~presynaptic process involved in chemical synaptic transmission	10	0.54	0.5511	65	1.11

Enrichment Score: 0.2380294607355356

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030324~lung development	17	0.91	0.5133	114	1.08
GO:0030323~respiratory tube development	17	0.91	0.5431	116	1.06
GO:0060541~respiratory system development	17	0.91	0.6930	127	0.97

Enrichment Score: 0.1378123959926619

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0090276~regulation of peptide hormone secretion	12	0.64	0.7146	90	0.96
GO:0002791~regulation of peptide secretion	12	0.64	0.7283	91	0.95
GO:0090087~regulation of peptide transport	12	0.64	0.7416	92	0.94

Enrichment Score: 0.13748100020737444

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0006606~protein import into nucleus	23	1.23	0.7262	176	0.95
GO:1902593~single-organism nuclear import	23	1.23	0.7262	176	0.95
GO:0044744~protein targeting to nucleus	23	1.23	0.7262	176	0.95
GO:0051170~nuclear import	23	1.23	0.7360	177	0.94

Table S3. Gene ontology functional enrichment analysis for genes upregulated in the associative pallium of *L. barbadensis*. All terms per cluster that were significant, had n > 10 and an enrichment score > 0.1. Bold p-values p < 0.05.

Enrichment Score: 3.9209423025572225

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0048666~neuron development	68	7.09	**0.0000**	550	1.70
GO:0031175~neuron projection development	58	6.05	**0.0000**	456	1.75
GO:0048699~generation of neurons	85	8.86	**0.0002**	792	1.48
GO:0030182~neuron differentiation	77	8.03	**0.0003**	710	1.49
GO:0022008~neurogenesis	86	8.97	**0.0010**	845	1.40

Enrichment Score: 2.388868893339189

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0007154~cell communication	266	27.74	**0.0008**	3124	1.17
GO:0023052~signalling	263	27.42	**0.0011**	3099	1.17
GO:0044700~single organism signalling	258	26.90	**0.0024**	3073	1.16
GO:0007165~signal transduction	236	24.61	**0.0066**	2836	1.15
GO:0051716~cellular response to stimulus	276	28.78	0.0823	3551	1.07

Enrichment Score: 2.3656249172309773

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0048812~neuron projection morphogenesis	38	3.96	**0.0027**	318	1.65
GO:0048667~cell morphogenesis involved in neuron differentiation	36	3.75	**0.0035**	301	1.65
GO:0061564~axon development	32	3.34	**0.0046**	262	1.68
GO:0007409~axonogenesis	30	3.13	**0.0078**	250	1.65

Enrichment Score: 2.054314434043748

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0051056~regulation of small GTPase mediated signal transduction	20	2.09	**0.0049**	138	2.00
GO:0046578~regulation of Ras protein signal transduction	18	1.88	**0.0052**	119	2.08
GO:0007265~Ras protein signal transduction	21	2.19	**0.0266**	174	1.66

Enrichment Score: 1.9718778925686844

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030334~regulation of cell migration	43	4.48	**0.0072**	394	1.50
GO:0040012~regulation of locomotion	46	4.80	**0.0073**	429	1.48
GO:2000145~regulation of cell motility	43	4.48	**0.0126**	408	1.45
GO:0051270~regulation of cellular component movement	45	4.69	**0.0195**	443	1.40

Enrichment Score: 1.8860648571231706

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0051960~regulation of nervous system development	49	5.11	**0.0034**	445	1.52
GO:0050767~regulation of neurogenesis	41	4.28	**0.0129**	385	1.47
GO:0060284~regulation of cell development	46	4.80	0.0508	486	1.30

Enrichment Score: 1.7779263001640087

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0031345~negative regulation of cell projection organization	15	1.56	**0.0019**	81	2.55
GO:0010977~negative regulation of neuron projection development	12	1.25	**0.0114**	70	2.36
GO:0045665~negative regulation of neuron differentiation	12	1.25	0.2185	116	1.43

Enrichment Score: 1.7472709685490513

Term	Count	%	PValue	Pop Hits	Fold Enrichment

GO:0006464~cellular protein modification process	173	18.04	**0.0016**	1942	1.23
GO:0036211~protein modification process	173	18.04	**0.0016**	1942	1.23
GO:0043412~macromolecule modification	175	18.25	**0.0092**	2049	1.18
GO:0044267~cellular protein metabolic process	195	20.33	0.2067	2547	1.06
GO:0019538~protein metabolic process	205	21.38	0.3622	2749	1.03

Enrichment Score: 1.6459431139047822

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030148~sphingolipid biosynthetic process	11	1.15	**0.0082**	58	2.61
GO:0006665~sphingolipid metabolic process	13	1.36	**0.0244**	88	2.04
GO:0046467~membrane lipid biosynthetic process	12	1.25	**0.0287**	80	2.07
GO:0006643~membrane lipid metabolic process	15	1.56	**0.0457**	118	1.75

Enrichment Score: 1.533645499677526

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0040013~negative regulation of locomotion	19	1.98	**0.0194**	147	1.78
GO:0030336~negative regulation of cell migration	16	1.67	**0.0282**	121	1.82
GO:2000146~negative regulation of cell motility	16	1.67	**0.0363**	125	1.76
GO:0051271~negative regulation of cellular component movement	18	1.88	**0.0369**	147	1.69

Enrichment Score: 1.4837611331217626

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0032872~regulation of stress-activated MAPK cascade	16	1.67	**0.0215**	117	1.88
GO:0070302~regulation of stress-activated protein kinase signalling cascade	16	1.67	**0.0230**	118	1.87
GO:0046328~regulation of JNK cascade	14	1.46	**0.0260**	99	1.95
GO:0032874~positive regulation of stress-activated MAPK cascade	12	1.25	**0.0287**	80	2.07

GO:0070304~positive regulation of stress-activated protein kinase signalling cascade	12	1.25	**0.0311**	81	2.04
GO:0046330~positive regulation of JNK cascade	11	1.15	**0.0339**	72	2.11
GO:0051403~stress-activated MAPK cascade	16	1.67	**0.0386**	126	1.75
GO:0031098~stress-activated protein kinase signalling cascade	16	1.67	0.0515	131	1.68
GO:0007254~JNK cascade	13	1.36	0.0571	100	1.79

Enrichment Score: 1.4758918987965997

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0010646~regulation of cell communication	140	14.60	**0.0176**	1629	1.18
GO:0023051~regulation of signalling	140	14.60	**0.0246**	1648	1.17
GO:0009966~regulation of signal transduction	121	12.62	0.0861	1476	1.13

Enrichment Score: 1.313928834415155

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0048489~synaptic vesicle transport	10	1.04	**0.0436**	65	2.12
GO:0097480~establishment of synaptic vesicle localization	10	1.04	**0.0436**	65	2.12
GO:0097479~synaptic vesicle localization	10	1.04	0.0601	69	2.00

Enrichment Score: 1.2674185516324012

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0007420~brain development	38	3.96	**0.0331**	375	1.40
GO:0007417~central nervous system development	45	4.69	0.0598	479	1.29
GO:0060322~head development	38	3.96	0.0798	403	1.30

Enrichment Score: 1.220880068382802

Term	Count	%	PValue	Pop Hits	Fold Enrichment

GO:0051961~negative regulation of nervous system development	19	1.98	**0.0279**	153	1.71
GO:0050768~negative regulation of neurogenesis	17	1.77	0.0513	142	1.65
GO:0010721~negative regulation of cell development	18	1.88	0.1521	179	1.39

Enrichment Score: 1.145148334137577

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0030838~positive regulation of actin filament polymerization	10	1.04	**0.0277**	60	2.30
GO:0008154~actin polymerization or depolymerisation	15	1.56	**0.0380**	115	1.80
GO:0030833~regulation of actin filament polymerization	13	1.36	**0.0383**	94	1.91
GO:0051258~protein polymerization	17	1.77	**0.0412**	138	1.70
GO:0008064~regulation of actin polymerization or depolymerisation	14	1.46	**0.0423**	106	1.82
GO:0030832~regulation of actin filament length	14	1.46	**0.0423**	106	1.82
GO:0032273~positive regulation of protein polymerization	10	1.04	0.0556	68	2.03
GO:0030041~actin filament polymerization	12	1.25	0.0680	92	1.80
GO:0032271~regulation of protein polymerization	13	1.36	0.0954	109	1.64
GO:0031334~positive regulation of protein complex assembly	10	1.04	0.4452	114	1.21
GO:0043254~regulation of protein complex assembly	16	1.67	0.5313	206	1.07

Table S4. Gene ontology functional enrichment analysis for genes upregulated in the associative pallium of *T. bicolor*. All terms per cluster that were significant, had n > 10 and an enrichment score > 0.1. Bold p-values p < 0.05.

Enrichment Score: 2.395152731488906

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0009123~nucleoside monophosphate metabolic process	25	2.76	**0.0001**	159	2.40
GO:0009126~purine nucleoside monophosphate metabolic process	23	2.54	**0.0002**	144	2.43
GO:0009199~ribonucleoside triphosphate metabolic process	23	2.54	**0.0002**	144	2.43
GO:0009161~ribonucleoside monophosphate metabolic process	23	2.54	**0.0004**	153	2.29
GO:0009167~purine ribonucleoside monophosphate metabolic process	22	2.43	**0.0004**	143	2.34
GO:0046034~ATP metabolic process	20	2.21	**0.0004**	124	2.46
GO:0009205~purine ribonucleoside triphosphate metabolic process	21	2.32	**0.0006**	138	2.32
GO:0009141~nucleoside triphosphate metabolic process	23	2.54	**0.0009**	162	2.16
GO:0009144~purine nucleoside triphosphate metabolic process	21	2.32	**0.0010**	143	2.24
GO:0009119~ribonucleoside metabolic process	25	2.76	**0.0020**	195	1.95
GO:0046128~purine ribonucleoside metabolic process	23	2.54	**0.0025**	176	1.99
GO:0042278~purine nucleoside metabolic process	23	2.54	**0.0027**	177	1.98
GO:0009116~nucleoside metabolic process	25	2.76	**0.0042**	206	1.85
GO:1901657~glycosyl compound metabolic process	25	2.76	**0.0080**	217	1.76
GO:0009259~ribonucleotide metabolic process	27	2.98	**0.0240**	264	1.56
GO:0019693~ribose phosphate metabolic process	27	2.98	**0.0333**	272	1.51
GO:0009150~purine ribonucleotide metabolic process	25	2.76	**0.0412**	252	1.51
GO:0006163~purine nucleotide metabolic process	25	2.76	0.0682	266	1.43
GO:0072521~purine-containing compound metabolic process	26	2.87	0.0908	288	1.38
GO:0009117~nucleotide metabolic process	30	3.31	0.1016	346	1.32
GO:0006753~nucleoside phosphate metabolic process	30	3.31	0.1216	353	1.30
GO:0055086~nucleobase-containing small molecule metabolic process	31	3.43	0.1789	385	1.23

Enrichment Score: 1.4669556555786873

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0043065~positive regulation of apoptotic process	27	2.98	**0.0296**	269	1.53
GO:0043068~positive regulation of programmed cell death	27	2.98	**0.0333**	272	1.51
GO:0010942~positive regulation of cell death	27	2.98	**0.0403**	277	1.49

Enrichment Score: 1.4208003282311281

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0098655~cation transmembrane transport	29	3.20	**0.0164**	280	1.58
GO:0098662~inorganic cation transmembrane transport	25	2.76	**0.0310**	245	1.56
GO:0098660~inorganic ion transmembrane transport	27	2.98	**0.0467**	281	1.46
GO:0034220~ion transmembrane transport	33	3.65	0.0872	381	1.32

Enrichment Score: 1.3407249450931762

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0019941~modification-dependent protein catabolic process	34	3.76	**0.0225**	351	1.48
GO:0006511~ubiquitin-dependent protein catabolic process	33	3.65	**0.0288**	345	1.46
GO:0043632~modification-dependent macromolecule catabolic process	34	3.76	**0.0297**	359	1.44
GO:0030163~protein catabolic process	45	4.97	**0.0317**	505	1.36
GO:0051603~proteolysis involved in cellular protein catabolic process	37	4.09	**0.0409**	408	1.38
GO:0044257~cellular protein catabolic process	38	4.20	0.0529	430	1.35
GO:0009057~macromolecule catabolic process	52	5.75	0.0977	649	1.22
GO:0044265~cellular macromolecule catabolic process	43	4.75	0.1454	543	1.21

Enrichment Score: 1.2378031650515533

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0034613~cellular protein localization	77	8.51	**0.0185**	920	1.28

GO:0070727~cellular macromolecule localization	77	8.51	**0.0215**	926	1.27
GO:0008104~protein localization	94	10.39	0.1085	1257	1.14
GO:0033036~macromolecule localization	100	11.05	0.2586	1419	1.07

Enrichment Score: 1.0975429658938938

Term	Count	%	PValue	Pop Hits	Fold Enrichment
GO:0032273~positive regulation of protein polymerization	11	1.22	**0.0125**	68	2.47
GO:0030838~positive regulation of actin filament polymerization	10	1.10	**0.0154**	60	2.54
GO:0008064~regulation of actin polymerization or depolymerisation	12	1.33	0.0863	106	1.73
GO:0030832~regulation of actin filament length	12	1.33	0.0863	106	1.73
GO:0030833~regulation of actin filament polymerization	11	1.22	0.0871	94	1.78
GO:0032271~regulation of protein polymerization	12	1.33	0.1003	109	1.68
GO:0008154~actin polymerization or depolymerisation	12	1.33	0.1321	115	1.59
GO:0030041~actin filament polymerization	10	1.10	0.1475	92	1.66
GO:0032535~regulation of cellular component size	16	1.77	0.5394	229	1.06

Tables S5. Module gene ontology terms of main functional annotation clusters. Functional annotation clusters that have the highest enrichment score for each module. Bold p-values $p < 0.05$.

Royalblue module	**Enrichment Score: 1.756**				
Term	GO number/Funct. Cat.	n	%	pvalue	Fold Enr.
cell junction	UP_KEYWORDS	6	8.96	**9E-04**	7.90
Synapse	UP_KEYWORDS	4	5.97	**0.009**	9.33
cell membrane	UP_KEYWORDS	7	10.45	**0.017**	3.31
ion transport	UP_KEYWORDS	5	7.46	**0.043**	3.74
ion channel	UP_KEYWORDS	4	5.97	0.063	4.32
Transport	UP_KEYWORDS	7	10.45	0.082	2.27

Skyblue module	**Enrichment Score: 1.148**				
Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
cellular respiration	GO:0045333	4	6.67	**0.007**	9.84
energy derivation by oxidation of organic compounds	GO:0015980	4	6.67	**0.024**	6.31
generation of precursor metabolites and energy	GO:0006091	4	6.67	0.059	4.39
oxidation-reduction process	GO:0055114	4	6.67	0.191	2.60
single-organism metabolic process	GO:0044710	8	13.33	0.923	0.75

Pink module	**Enrichment Score: 0.806**				
Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
regulation of cell migration	GO:0030334	10	9.09	**0.008**	2.86
regulation of cell motility	GO:2000145	10	9.09	**0.009**	2.75
negative regulation of cell migration	GO:0030336	4	3.64	**0.013**	8.07
regulation of locomotion	GO:0040012	10	9.09	**0.013**	2.61
regulation of cellular component movement	GO:0051270	10	9.09	**0.016**	2.53
negative regulation of cell migration	GO:0030336	5	4.55	**0.024**	4.52
negative regulation of cell motility	GO:2000146	5	4.55	**0.027**	4.37
reg. of protein modif. by small protein conjugation	GO:1903320	5	4.55	**0.027**	4.33
negative regulation of locomotion	GO:0040013	5	4.55	**0.044**	3.72
negative regulation of cellular component movement	GO:0051271	5	4.55	**0.044**	3.72
positive regulation of endothelial cell migration	GO:0010595	3	2.73	0.054	7.86

cell motility	GO:0048870	12	10.91	0.064	1.79
localization of cell	GO:0051674	12	10.91	0.065	1.79
cell migration	GO:0016477	11	10.00	0.075	1.82
regulation of catalytic activity	GO:0050790	13	11.82	0.103	1.60
regulation of transferase activity	GO:0051338	8	7.27	0.11	1.95
regulation of protein kinase activity	GO:0045859	7	6.36	0.111	2.10
positive regulation of epithelial cell migration	GO:0010634	3	2.73	0.113	5.16
regulation of localization	GO:0032879	17	15.45	0.123	1.43
Locomotion	GO:0040011	12	10.91	0.126	1.58
regulation of endothelial cell migration	GO:0010594	3	2.73	0.128	4.77
positive regulation of cell migration	GO:0030335	5	4.55	0.138	2.48
positive regulation of cell motility	GO:2000147	5	4.55	0.145	2.44
positive regulation of locomotion	GO:0040017	5	4.55	0.155	2.37
positive regulation of cellular component movement	GO:0051272	5	4.55	0.155	2.37
regulation of kinase activity	GO:0043549	7	6.36	0.16	1.89
regulation of molecular function	GO:0065009	15	13.64	0.168	1.40
negative regulation of cellular metabolic process	GO:0031324	14	12.73	0.177	1.41
negative regulation of cell proliferation	GO:0008285	4	3.64	0.206	2.53
endothelial cell migration	GO:0043542	3	2.73	0.212	3.46
regulation of anatomical structure morphogenesis	GO:0022603	8	7.27	0.225	1.60
movement of cell or subcellular component	GO:0006928	12	10.91	0.238	1.38
regulation of epithelial cell migration	GO:0010632	3	2.73	0.243	3.15
negative regulation of macromolecule metabolic process	GO:0010605	13	11.82	0.246	1.34
negative regulation of metabolic process	GO:0009892	14	12.73	0.248	1.32
positive regulation of developmental process	GO:0051094	8	7.27	0.282	1.49
ameboidal-type cell migration	GO:0001667	4	3.64	0.289	2.11
neg. Reg. of nucleobase-cont. compound metab. process	GO:0045934	8	7.27	0.298	1.46
negative regulation of cell proliferation	GO:0008285	5	4.55	0.348	1.66
epithelial cell migration	GO:0010631	3	2.73	0.371	2.30
epithelium migration	GO:0090132	3	2.73	0.382	2.25
regulation of cell adhesion	GO:0030155	5	4.55	0.391	1.56
tissue migration	GO:0090130	3	2.73	0.395	2.18

negative regulation of nitrogen compound metabolic process	GO:0051172	8	7.27	0.406	1.30
negative regulation of transcription	GO:0045892	3	2.73	0.425	2.06
regulation of binding	GO:0051098	3	2.73	0.428	2.04
regulation of multicellular organismal development	GO:2000026	10	9.09	0.469	1.17
negative regulation of RNA metabolic process	GO:0051253	6	5.45	0.511	1.26
positive regulation of multicellular organismal process	GO:0051240	8	7.27	0.545	1.14
negative regulation of cellular biosynthetic process	GO:0031327	7	6.36	0.556	1.16
negative regulation of biosynthetic process	GO:0009890	7	6.36	0.567	1.15
neg. reg. of cellular macromolecule biosynthetic process	GO:2000113	6	5.45	0.638	1.09
negative regulation of transcription	GO:0045892	5	4.55	0.652	1.12
negative regulation of nucleic acid-templated transcription	GO:1903507	5	4.55	0.655	1.11
negative regulation of RNA biosynthetic process	GO:1902679	5	4.55	0.668	1.09
negative regulation of macromolecule biosynthetic process	GO:0010558	6	5.45	0.677	1.05
negative regulation of gene expression	GO:0010629	6	5.45	0.728	0.99

Salmon module	**Enrichment Score: 1.003**				
Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
adult locomotory behaviour	GO:0008344	7	7.78	**0.007**	22.15
adult behaviour	GO:0030534	4	4.44	**0.028**	6.01
Behaviour	GO:0007610	6	6.67	0.064	2.74
single-organism behaviour	GO:0044708	5	5.56	0.065	3.24
locomotory behaviour	GO:0007626	4	4.44	0.071	4.11
central nervous system development	GO:0007417	7	7.78	0.101	2.15
hindbrain development	GO:0030902	3	3.33	0.104	5.38
neuron projection development	GO:0031175	6	6.67	0.192	1.92
brain development	GO:0007420	5	5.56	0.245	1.95
nervous system development	GO:0007399	11	12.22	0.266	1.36
head development	GO:0060322	5	5.56	0.285	1.82
neuron development	GO:0048666	6	6.67	0.309	1.60
cell projection organization	GO:0030030	6	6.67	0.548	1.21

Lightgreen module	**Enrichment Score: 1.671**				
Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.

positive regulation of biological process	GO:0048518	29	39.73	**2E-04**	1.88
regulation of cellular process	GO:0050794	42	57.53	**3E-04**	1.43
regulation of biological process	GO:0050789	43	58.90	**5E-04**	1.40
biological regulation	GO:0065007	44	60.27	**0.001**	1.33
positive regulation of cellular process	GO:0048522	25	34.25	**0.002**	1.79
single organism signalling	GO:0044700	27	36.99	**0.004**	1.62
Signalling	GO:0023052	27	36.99	**0.005**	1.60
cell communication	GO:0007154	27	36.99	**0.005**	1.59
signal transduction	GO:0007165	25	34.25	**0.006**	1.62
intracellular signal transduction	GO:0035556	16	21.92	**0.009**	1.97
cellular response to stimulus	GO:0051716	27	36.99	**0.031**	1.40
response to stimulus	GO:0050896	30	41.10	0.054	1.29
regulation of response to stimulus	GO:0048583	16	21.92	0.071	1.54
single-organism cellular process	GO:0044763	42	57.53	0.073	1.15
regulation of cell communication	GO:0010646	14	19.18	0.085	1.57
regulation of signalling	GO:0023051	14	19.18	0.091	1.56
single-organism process	GO:0044699	45	61.64	0.104	1.11
regulation of signal transduction	GO:0009966	12	16.44	0.156	1.49
cellular process	GO:0009987	47	64.38	0.735	0.99
primary metabolic process	GO:0044238	25	34.25	0.919	0.86
cellular metabolic process	GO:0044237	25	34.25	0.942	0.84
metabolic process	GO:0008152	27	36.99	0.957	0.84

Brown module **Enrichment Score: 5.040**

Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
cellular component organization or biogenesis	GO:0071840	74	45.12	**6E-07**	1.60
cellular component organization	GO:0016043	68	41.46	**2E-05**	1.53
organelle organization	GO:0006996	47	28.66	**7E-05**	1.73

White module **Enrichment Score: 1.256**

Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
plasma membrane organization	GO:0007009	5	8.47	**0.01**	5.84
single-organism cellular localization	GO:1902580	8	13.56	**0.016**	2.92

endomembrane system organization	GO:0010256	6	10.17	**0.017**	3.87
protein localization to plasma membrane	GO:0072659	4	6.78	**0.024**	6.28
cellular protein localization	GO:0034613	10	16.95	**0.028**	2.22
protein localization to cell periphery	GO:1990778	4	6.78	**0.029**	5.86
cellular macromolecule localization	GO:0070727	10	16.95	**0.029**	2.21
cellular localization	GO:0051641	12	20.34	**0.049**	1.82
protein localization to membrane	GO:0072657	4	6.78	0.095	3.59
Transport	UP_KEYWORDS	6	10.17	0.131	2.19
single-organism membrane organization	GO:0044802	5	8.47	0.145	2.41
regulation of cellular localization	GO:0060341	5	8.47	0.171	2.25
membrane organization	GO:0061024	5	8.47	0.199	2.12
organic substance transport	GO:0071702	7	11.86	0.573	1.13

Red module	**Enrichment Score: 2.171**				
Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
receptor localization to synapse	GO:0097120	6	5.31	**9E-04**	112.77
protein localization to synapse	GO:0035418	3	2.65	**0.004**	30.76
protein localization to membrane	GO:0072657	8	7.08	**0.005**	3.75
receptor clustering	GO:0043113	3	2.65	**0.02**	13.37
protein complex localization	GO:0031503	4	3.54	**0.026**	6.21
localization within membrane	GO:0051668	3	2.65	**0.049**	8.31

Turquoise module	**Enrichment Score: 3.731**				
Term	GO number/Funct. Cat.	n	%	PValue	Fold Enr.
cellular component organization or biogenesis	GO:0071840	209	34.15	**1E-04**	1.23
organelle organization	GO:0006996	133	21.73	**2E-04**	1.33
cellular component organization	GO:0016043	200	32.68	**3E-04**	1.22

Table S6. Mean expression of each glutamate receptor in each region, using *in situ* hybridization data. Values are the means of optical densities (OD) of all individuals for every receptor in each brain region, per species. Values in bold indicate significant differences between species in a given region following ANOVA and Bonferroni interspecific post-test for each region and each receptor. Bold p-values $p < 0.05$.

		GRIN1			GRIN2A			GRIN2B			GRIN2C			GRIN3		
		BF	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***
NMDA receptors	Arcopallium	39.6	23.9	0.1145	2.8	5.7	0.4020	10.9	9.8	0.8324	4.08	4.03	0.8711	9.2	5.2	0.3499
	Entopallium	44.8	34.8	0.3087	14.4	19.0	0.2182	8.4	7.7	0.8924	4.07	4.27	0.5558	4.1	3.9	0.9586
	Hippocampus	74.7	42.2	**0.0036**	4.2	5.9	0.6242	16.5	9.6	0.2340	4.94	4.73	0.5554	21.4	19.8	0.7212
	Mesopallium	79.3	56.7	**0.0251**	15.2	22.0	0.0525	30.9	15.4	**0.0043**	4.94	4.50	0.1820	17.5	12.0	0.2385
	Nidopallium	71.5	45.2	**0.0101**	8.8	13.8	0.1548	25.2	10.8	**0.0074**	4.61	4.32	0.3683	15.2	11.5	0.4217
	NCL	62.9	46.0	0.0892	6.8	12.1	0.1292	29.2	12.5	**0.0023**	4.02	4.04	0.9531	15.1	13.8	0.7858
		GRM1			**GRM2**			**GRM3**			**GRM4**			**GRM5**		
		BF	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***
Metabotropic	Arcopallium	3.1	3.8	0.8429	5.1	15.8	0.0835	23.6	23.9	0.9454	24.3	35.2	0.6658	2.0	3.6	0.2461
	Entopallium	11.6	11.6	0.9952	3.5	14.0	0.0880	29.5	26.0	0.4435	50.0	74.3	0.3377	4.3	6.8	0.1013
	Hippocampus	10.4	5.1	0.1343	12.2	24.0	0.0745	15.5	13.9	0.6460	32.3	26.3	0.8139	4.0	5.3	0.3627
	Mesopallium	9.9	10.1	0.9604	25.5	56.5	**0.0001**	26.4	24.6	0.6880	31.9	36.2	0.8932	6.4	7.7	0.3361
	Nidopallium	11.1	10.8	0.9414	23.8	54.3	**0.0001**	27.3	27.4	0.9789	24.5	29.2	0.8837	6.0	7.8	0.2022
	NCL	7.8	10.8	0.4019	14.8	38.7	**0.0003**	25.2	33.0	0.0854	13.8	26.7	0.6833	8.0	8.3	0.8311
		GRIK1			**GRIK2**			**GRIK3**			**GRIK4**					
		BF	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***			
Kainate receptors	Arcopallium	10.5	8.9	0.7497	11.4	15.2	0.3148	12.3	12.4	0.9836	5.9	11.9	0.5965			
	Entopallium	13.3	16.9	0.4591	18.9	15.7	0.3987	23.5	18.0	0.2660	24.7	46.3	**0.0405**			
	Hippocampus	21.8	14.0	0.1167	13.8	12.1	0.6576	8.8	9.5	0.9018	17.8	5.8	0.2415			
	Mesopallium	13.1	13.0	0.9857	18.7	24.9	0.1338	21.5	22.5	0.8337	20.2	25.0	0.6343			
	Nidopallium	12.0	13.3	0.7858	11.0	15.5	0.2649	14.2	13.9	0.9417	13.9	20.1	0.5364			
	NCL	9.6	11.1	0.7648	13.7	15.6	0.6712	11.9	12.0	0.9840	17.5	19.1	0.8712			
		GRIA1			**GRIA2**			**GRIA3**			**GRIA4**					
		BF	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***			
AMPA receptors	Arcopallium	15.2	15.9	0.8314	35.4	29.3	0.0700	29.2	26.7	0.7116	11.9	15.9	0.3718			
	Entopallium	16.8	17.6	0.8199	36.6	36.4	0.9436	37.9	36.5	0.8392	45.2	40.9	0.3736			
	Hippocampus	28.6	27.8	0.8292	41.9	39.2	0.4031	28.0	28.1	0.9849	18.0	14.0	0.3724			
	Mesopallium	21.7	26.1	0.1862	42.6	39.5	0.4262	33.6	32.0	0.8152	32.0	32.0	0.9945			
	Nidopallium	15.0	14.3	0.8723	37.5	35.5	0.5892	26.9	26.1	0.9202	29.2	28.9	0.9434			
	NCL	14.5	14.8	0.9172	38.1	34.6	0.3515	33.4	31.7	0.7953	28.1	33.3	0.2440			

Table S7. Mean expression of each glutamate receptor in each region, using RNA-Seq data. Values are the means of transformed read data (variance stabilizing transformation) of all individuals for every receptor in each brain region, per species. Values in bold indicate significant differences of transformed p-values following RNA-Seq analysis between species in a given region. Bold p-values $p < 0.05$.

		GRIN1			GRIN2A			GRIN2B			GRIN2C			GRIN3		
NMDA receptors		**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***
	Arcopallium	14.1	13.7	0.5068	7.3	8.1	0.1205	8.5	8.0	0.2239	5.47	4.75	0.1217	9.4	9.3	0.7657
	Entopallium	14.5	14.4	0.9715	8.9	9.7	**0.0089**	7.5	7.5	0.9782	4.88	4.74	0.6393	8.7	8.1	0.1025
	Hippocampus	14.2	13.8	0.2709	7.7	7.9	0.9912	8.8	8.4	0.2123	5.94	5.54	0.3917	10.2	9.9	0.6603
	Mesopallium	14.0	13.8	0.4299	8.4	8.7	0.2981	9.1	8.6	**0.0108**	5.97	5.59	0.0941	9.4	9.2	0.6166
	Nidopallium	14.1	13.8	0.0572	8.5	8.9	0.1519	9.5	9.0	**0.0340**	5.59	5.83	0.7103	9.5	9.2	0.1517
	NCL	14.1	13.7	0.0535	8.1	8.8	**0.0030**	8.7	8.4	0.3480	5.24	5.11	0.1730	9.6	9.2	0.0830
		GRM1			**GRM2**			**GRM3**			**GRM4**			**GRM5**		
Metabotropic		**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***
	Arcopallium	7.6	7.5	0.8917	9.8	10.1	0.5758	11.4	11.7	0.6087	10.7	10.9	0.7591	6.1	6.3	0.8807
	Entopallium	6.9	6.8	0.9524	8.8	8.7	0.9421	10.8	11.2	**0.0415**	11.2	11.8	**0.0085**	6.0	6.4	0.3465
	Hippocampus	7.8	7.4	0.7719	9.8	10.0	0.8738	11.2	11.5	0.6603	10.5	10.4	0.9341	6.4	6.4	0.9568
	Mesopallium	7.4	7.2	0.6940	10.0	10.5	**0.0069**	11.0	11.4	**0.0132**	11.3	11.6	0.0882	6.7	6.6	0.7593
	Nidopallium	8.0	7.8	0.6097	9.9	10.4	**0.2116**	11.2	11.5	**0.0066**	10.8	11.3	**0.0033**	7.4	7.1	0.6781
	NCL	7.6	7.6	0.9983	10.0	10.2	0.6116	11.4	11.7	**0.0171**	11.0	11.1	0.2124	6.4	6.6	0.9529
		GRIK1			**GRIK2**			**GRIK3**			**GRIK4**					
Kainate receptors		**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***			
	Arcopallium	6.9	7.3	0.5745	7.2	7.1	0.9630	7.6	7.5	0.8676	7.7	7.5	0.8145			
	Entopallium	9.1	9.2	0.9240	6.4	7.0	0.5890	8.5	8.3	0.8698	7.6	7.8	0.9371			
	Hippocampus	7.9	7.7	0.6579	7.1	7.1	0.9665	7.9	7.9	0.9806	8.2	7.9	0.6621			
	Mesopallium	6.9	7.0	0.7875	8.1	7.8	0.3087	8.5	8.4	0.7513	8.7	8.6	0.9765			
	Nidopallium	7.3	7.4	0.6357	7.9	7.7	0.6302	7.7	8.0	0.5867	8.6	9.0	**0.0321**			
	NCL	6.8	7.3	0.1062	7.4	7.3	0.6648	7.7	7.8	0.9176	8.5	8.4	0.8213			
		GRIA1			**GRIA2**			**GRIA3**			**GRIA4**					
AMPA receptors		**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***	**BF**	**GQ**	***p***			
	Arcopallium	9.3	8.9	0.5916	11.2	11.4	0.9035	10.7	10.7	0.9997	10.0	10.4	0.5113			
	Entopallium	8.4	8.6	0.7554	11.3	11.3	0.8896	9.5	9.4	0.8846	8.5	8.6	0.9147			
	Hippocampus	9.3	9.3	0.9207	11.3	11.4	0.9798	10.4	10.3	0.9351	10.0	9.9	0.9380			
	Mesopallium	9.7	9.5	0.4907	11.6	11.5	0.9271	10.5	10.3	0.5815	10.4	10.3	0.7403			
	Nidopallium	9.1	8.9	0.2280	11.4	11.4	0.9872	10.4	10.4	0.9661	10.5	10.5	0.7211			
	NCL	8.8	8.6	0.6677	11.4	11.5	0.5082	10.5	10.5	0.9825	10.3	10.7	0.0880			

Table S8. Glutamate receptor nucleotide identities. Identities for individual nucleotide correspondence of whole coding DNA sequences (cds) for each neurotransmitter receptor, comparing both study species with *G. gallus*, and between study species. Sequences for *L. barbadensis* and *T. bicolor* were obtained from this study's RNA-Seq data.

					Identities		
	Gene	**G. Gallus Gene ID**	**G. Gallus Transcript ID**	**Cds length**	***L. barbadensis* vs *G. gallus***	***T. bicolor* vs *G. gallus***	***L. barbadensis* vs *T. bicolor***
NMDA	GRIN1	ENSGALG00000008898	ENSGALT00000040369	2898	2720/2898 (93.9%)	2723/2898 (94%)	2880/2898 (99.4%)
	GRIN2A	ENSGALG00000007278	ENSGALT00000011793	4377	4017/4377 (91.8%)	4014/4377 (91.7%)	4364/4377 (99.7%)
	GRIN2B	ENSGALG00000011809	ENSGALT00000019272	4515	4293/4515 (95.1%)	4298/4515 (95.2%)	4496/4512 (99.6%)
	GRIN2C	ENSGALG00000027415	ENSGALT00000044113	3174	2782/3164 (87.9%)	2571/2923 (88%)	2856/2913 (98.0%)
	GRIN3A	ENSGALG00000015551	ENSGALT00000025070	3270	2928/3279 (89.3%)	2907/3247 (89.5%)	3198/3209 (99.7%)
Metabotropic	GRM1	ENSGALG00000012297	ENSGALT00000020095	3459	3013/3414 (88.3%)	3022/3414 (88.5%)	3378/3407 (99.1%)
	GRM2	ENSGALG00000003839	ENSGALT00000006095	2613	2358/2613 (90.2%)	2182/2458 (88.8%)	2333/2458 (94.9%)
	GRM3	ENSGALG00000006576	ENSGALT00000010624	2670	2399/2670 (89.9%)	2395/2670 (89.7%)	2648/2670 (99.2%)
	GRM4	ENSGALG00000002840	ENSGALT00000004481	2748	2520/2724 (92.5%)	2528/2723 (92.8%)	2705/2724 (99.3%)
	GRM5	ENSGALG00000017238	ENSGALT00000027866	3729	3435/3736 (91.9%)	3441/3736 (92.1%)	3712/3735 (99.4%)
Kainate	GRIK1	ENSGALG00000015835	ENSGALT00000025530	2580	2041/2431 (84%)	2116/2446 (86.5%)	2249/2397 (93.8%)
	GRIK2	ENSGALG00000015434	ENSGALT00000024893	2649	2405/2562 (93.9%)	2410/2562 (94.1%)	2550/2562 (99.5%)
	GRIK3	ENSGALG00000002098	ENSGALT00000003274	2667	2382/2671 (89.2%)	2372/2646 (89.6%)	2558/2647 (96.6%)
	GRIK4	ENSGALG00000006638	ENSGALT00000045454	2277	2021/2262 (89.3%)	1958/2183 (89.7%)	2176/2188 (99.5%)
AMPA	GRIA1	ENSGALG00000004083	ENSGALT00000006493	2709	2387/2712 (88%)	2480/2709 (91.5%)	2565/2712 (94.6%)
	GRIA2	ENSGALG00000009405	ENSGALT00000038841	2652	2535/2652 (95.6%)	2534/2652 (95.6%)	2648/2652 (99.8%)
	GRIA3	ENSGALG00000008512	ENSGALT00000013864	2667	2406/2669 (90.1%)	2490/2667 (93.4%)	2529/2669 (94.8%)
	GRIA4	ENSGALG00000017178	ENSGALT00000036434	2709	2588/2709 (95.5%)	2597/2709 (95.9%)	2696/2709 (99.5%)

General Conclusion

In the four chapters of this thesis, I employed a series of methods from behavioral ecology, comparative psychology and neuroscience to study innovativeness in birds. In brief, I found that i) obstacle-removal problem-solving tasks in captivity reflect innovativeness in wild birds, ii) problem-solving ability is increased with urbanization and iii) innovativeness is associated with an increase in the activity of specific genes in the associative pallium and differentially expressed glutamate receptors.

In Chapter 1, we documented a previously reported innovation of *L. barbadensis* (Reader et al. 2002) and we assessed its diffusion. We showed that the sugar packet innovation appeared independently at least three times in Barbados as well as in St. Lucia in the ancestral species of L. barbadensis, *Loxigilla noctis*. In addition, since the publication of Chapter 1, the sugar packet innovation has been observed in *L. barbadensis* at a fourth distant site in Barbados at Crane Beach, 20 kilometers away from the closest previously observed innovation site (Dr Grete Pasch, pers. comm., 2014). This provides further evidence that the same innovation appeared independently multiple times in *L. barbadensis*. Noisy miners (*Manorina melanocephala*), which are known for their highly innovative nature in urban environments (Griffin and Diquelou 2015), were also later observed performing the same sugar packet opening innovation in Australia (Delgado and Correa 2015). The classical milk bottle opening innovation (Fisher and Hinde 1949) shows evidence of both independent appearance (Sherry and Galef 1984; Lefebvre 1995) and cultural transmission (Aplin et al. 2013). Our results on Barbados bullfinches suggest that in this particular case, most of the innovations have occurred independently.

In Chapter 2, we have shown clear differences in problem-solving skills between urban and rural *L. barbadensis*, using two different obstacle-removal tasks. This is in accordance with our prediction that where innovation opportunities increase (e.g. presence of sugar packets), innovativeness is favored. The relationship between urbanization and problem-solving was recently corroborated by a study by Preiszner et al. (2017), which also reported higher problem-solving skills in urban great tits compared to rural ones. On the other hand, another study by Federspiel et al. (2017)

found that urban common mynas needed more trials than rural ones to succeed in associative learning tasks. This contrasts with our results on the performance of *L. barbadensis* in associative learning tasks, which did not differ between urban and rural populations. In addition, we did not find any significant interspecific difference in acquisition nor in reversal learning skills in the study of Chapter 4, suggesting that those skills are not related to innovation. As discussed in Appendix II, the bulk of the evidence in birds suggests that reversal learning and problem-solving measure different cognitive abilities.

We have shown in Chapter 3 that *L. barbadensis* is capable of solving the famous string-pulling task with one of the best scores of all species ever tested (when data are available and *n* is sufficiently large, see Jacobs and Osvath 2015). It is puzzling that performance on the string-pulling task does not correlate with other problem-solving tasks at the inter-individual level, while the performance in other problem-solving tasks does correlate (Chapter 2). The string-pulling task differs from the three other problem-solving tasks that we presented to our *Thraupidae* in the fact that it is not an obstacle-removal task. It would be interesting to test conjointly other tasks that are similar in nature to the string-pulling task to isolate the cognitive skill required for this task.

The project described in Chapter 4 pinpoints for the first time neuromolecular correlates of innovative problem-solving abilities in birds. The divergence that we observed in glutamate receptors, especially the GRIN2B/GRIN2A ratio, is extremely robust, since the same result was obtained using three different methods. Nonetheless, this observation was made on only two species. Other factors besides innovativeness differences might be behind the divergence between *L. barbadensis* and *T. bicolor*. However, the divergence in innovativeness between the two sister-species is striking, and the absence of differences for other traits, except for risk-taking behaviors, argues against the possibility that the neuromolecular variation reflects a divergence in another cognitive trait. While we cannot exclude the possibility that the differentially expressed glutamate receptors are related to risk-taking behavior differences instead of problem-solving skills, it would be against the large body of literature that link glutamate receptors with cognition, and not risk-taking behaviors. Roth and colleagues (2010;

2012) have shown that differences in a risk-taking neophobia test between northerly and southerly populations of black-capped chickadees are associated with differences in the arcopallium, an avian brain area that is the presumed equivalent to the mammalian amygdala, but in which we found very few difference between our two Barbadian finches. Finally, because the NCL is a region known to be involved in complex cognition, and not personality, the noticeable change in gene expression that we found in this region is more likely due to the divergence in innovativeness that characterize the two *Thraupidae*.

The diversity of tasks I have employed in the different projects presented in this thesis leads to interesting comparisons among them. First, the relationship between performances on the different obstacle-removal problem-solving tasks is coherent. They correlate with each other (Chapter 2) and vary with innovativeness, at interspecific (Chapter 4) and interpopulational (Chapter 2) levels. This further confirms that obstacle-removal problem-solving tasks should be the method of choice to measure innovativeness in captivity (Griffin and Guez 2014). Surprisingly, however, obstacle-removal ability was not associated with string-pulling ability, which also is independent from all other tested behavioral tasks (Chapter 3). More research will be needed to learn which are the traits that influence string-pulling ability, if there are any. Interestingly, associative learning skills turn out to be independent of problem-solving skills. I provide robust evidence that performance on both acquisition and reversal learning tasks is not associated with any problem-solving tasks at the intraspecific (Chapter 2, Chapter 3 and Chapter 4), interpopulational (Chapter 2) and interspecific (Chapter 4) levels. Correspondingly, associative learning skills do not appear to be related to innovativeness at any level, at least in our tested species. This absence of association further supports our postulate that associative learning and innovativeness are distinct abilities and as a consequence they should not be designated under the same umbrella term "behavioral flexibility" (Appendix II). Finally, our results suggest that there is no general cognitive ability (i.e. *g*) that determines, even in part, performance on all behavioral tasks, either at the intraspecific or interspecific levels. This contrasts with the results obtained in primates, for which there is strong evidence supporting the existence of a *g* factor influencing several behavioral traits (Reader et al. 2011). This surprising difference

could reside in the fact that there are many more species of bird than there are of primates, which could encompass a greater diversity of cognitive "syndromes". Birds also live in a wider diversity of habitats. In any case, further research should focus on the reasons behind the discrepancies in the associations between cognitive traits observed in the different taxa.

As far as temperament in concerned, we found that boldness was higher in *L. barbadensis* than in *T. bicolor*, and higher in urban than rural *L. barbadensis*. Although bolder population/species are also more innovative, there was no correlation between boldness and problem-solving among individuals of a given species or population (Chapter 2, Chapter 3 and Chapter 4). This suggests that different contingencies cohabit to favor boldness and innovativeness at the same time, but that innovation capacity is not necessarily related to boldness. Neophobia also varied at interspecific and interpopulational levels, although in opposite directions. Similar to boldness, it was not associated with problem-solving among individuals. Our results are against the view that risk-taking behaviors are connected with innovation (e.g. Sol et al. 2012; but see Griffin and Guez 2014). Some personality traits can probably facilitate innovation in some contexts, but in the light of our results, they are not directly linked with variation in innovativeness.

In sum, the discoveries in this thesis were made possible because we used the neuroecological approach. In contrast, our results would not have been as clear if we had tackled our questions solely using the psychological approach. First, we chose a species that is specialized in the behavior of interest, innovativeness. If we had instead opted for a Phasianid, for example, we would probably not have detected variation in problem-solving skills between birds living in rural and urban areas, just like the van Horik and Madden (2016) study that failed to detect any meaningful variation between pheasant chicks.

Secondly, our tasks were chosen specifically to represent the behavior of interest, innovativeness. If we had chosen only a classical psychology task as a proxy for general cognitive abilities, for example reversal learning, we would have come to the conclusion that rural and urban populations do not differ (reversal learning results in Chapter 2),

and that our two species of *Thraupidae* are cognitively similar (reversal learning results in Chapter 4), which is in complete disagreement with the observed divergent levels of innovation that differentiate those species in the wild (Chapter 1 and Appendix III).

Future directions

As is typical in ecology, more studies with different species will be required to confirm our results. The relationships – and the equally interesting absence of relationships – in performance on different tasks should be assessed in more species from the wild to draw general conclusions, as it is likely that different environmental contingencies select for different combinations of abilities. Comparing abilities of more species living in urbanized and non-urbanized areas could also reveal which species have the capacity to react to human perturbations, and possibly unveil different strategies to do so, if being innovative is not the only one. Similarly, research on different species will be needed to confirm our findings on the neural bases of innovation. Our pair of sister species is a unique research model that allowed us to reveal a neuromolecular pattern behind their extreme divergence in innovativeness and problem-solving. Yet, finding the same neural differences in other species that diverge in the same way would strengthen our conclusions. It would also be enlightening to conduct studies with species that differ in other traits, but not in innovativeness. This could help clarify the exact implication of the NCL in different cognitive abilities. In fact, our findings in the NCL appear to be in conflict with some of the previous results in the literature. We found an association between problem-solving and gene expression patterns in the NCL. However, many of the previous investigations on the NCL used classical psychology tasks like associative learning (e.g. Diekamp et al. 2000; Herold 2010). If neuronal activity in the NCL is linked with associative learning skills, we should have observed an increase of this skill in *L. barbadensis* compared to *T. bicolor*, but it was not the case. However, it is important to note that previous results on the NCL were all obtained using intraspecific comparisons, preventing any discovery of a specialization related to a specific task. In the light of this fact, the NCL could be involved in basic associative learning, but complex problem-solving could require a specialization of the NCL for higher processing skills. There might also be a sub-regional compartmentalization of the NCL as is known to be the case for the PFC, with distinct areas of the NCL being specialized

in different skills. Again, additional research, ideally involving a variety of species and tasks, will help disentangle those fundamental issues. In addition, it would be of great interest to manipulate the genes that we found to be associated with innovation. Modulating the GRIN2B/GRIN2A ratio, for instance, could be achieved by pharmacological approaches or using viral vectors that specifically target GRIN2B and GRIN2A expression separately. Then, evaluating problem-solving skills, along with other cognitive skills and temperament traits to assess the specificity of the treatment, would help determine the causal role of NMDA receptors in problem-solving. We could also eventually aim at increasing the innovativeness of a conservative bird and decreasing that of an opportunistic one, similar to the experiments that have been done on mice.

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Appendices

Connecting statement

The following three papers are included as appendices because they are only indirectly relevant to the main theme of the thesis. Appendix I describes the molecular sexing of the monomorphic *L. barbadensis.* Appendix II is an opinion piece on the use of the term 'behavioral flexibility' in animal cognition. Appendix III is a review of innovativeness in the superfamily to which *L. barbadensis* and *T. bicolor* belong. It is included as an appendix rather than a full-fledged chapter because I am not sole or co- first author on the publication.

Appendix I.

Morphological and Molecular Sexing of the Monochromatic Barbados Bullfinch Loxigilla barbadensis

Audet J.-N., Ducatez S. and Lefebvre L.

Citation: Audet J-N, Ducatez S, Lefebvre L. 2014. Morphological and molecular sexing of the monochromatic Barbados bullfinch, Loxigilla barbadensis. Zoological Science. 31:687–691.

Abstract

The bullfinch *Loxigilla barbadensis* is an endemic passerine on the Caribbean island of Barbados that has only recently been taxonomically split from the Lesser Antillean bullfinch *L. noctis.* The trait that most clearly distinguishes *L. barbadensis* from *L. noctis* is the absence in the male of sexually dimorphic coloration of the body and throat feathers, with *L. barbadensis* males and females sharing the same dull brown plumage. Here we report, in 64 individuals netted throughout the island, the results of a discriminant analysis on two (wing length and tail length) to four morphological traits showing very high (97%) concordance with sexing via PCR taken from blood. Females also show a paler lower mandible, a trait that yields an 80% concordance with PCR sexing. We found one *L. barbadensis* male that had a *noctis*-like reddish throat patch, supporting the idea that sexual dichromatism is the ancestral condition and that male Barbados bullfinches have evolved cryptic coloration that now makes the species monochromatic.

Introduction

The bullfinch *Loxigilla barbadensis* is an endemic passerine on the Caribbean island of Barbados. Until 2004, it was considered a subspecies of the Lesser Antillean bullfinch *L. noctis*, but it has since been elevated to species status (Buckley and Buckley, 2004; Gill and Donsker, 2014). The trait that most clearly distinguishes *L. barbadensis* from *L. noctis* is the absence in the male of sexually dimorphic coloration of the body (black) and throat (red) feathers, with *L. barbadensis* males and females sharing the same dull brown plumage. This makes sexing of Barbados bullfinches very difficult.

Several field and captive behavioral studies of this species have been conducted, including work on parasites (Fallon et al., 2003; Svensson-Coelho and Ricklefs, 2011) and various aspects of cognition and personality (Webster and Lefebvre, 2000, 2001; Reader et al., 2002; Ducatez et al., 2013). Research on other passerines shows that sex plays a key role in, among many other traits, learning and parasitism. For instance, in great tits, Dunn et al. (2011) showed a strong interaction between sex and the effects of malaria parasites on problem-solving, exploration, and risk-aversion, while Brust et al. (2013) found that male zebra finches are better at reversal learning than females. Sexing the monomorphic Barbados bullfinch is thus important for ongoing field work, but studies to date have overlooked this potential variable. The purpose of this study is to provide an easy tool for sexing Barbados bullfinches in the field.

Buckley and Buckley (2004) have suggested that male Barbados bullfinches display a "duskier" lower mandible than females, although birding guides (e.g., Evans, 1990; Bond, 1993; Raffaele et al., 2010) and articles (e.g., Bond, 1979) qualify the species as sexually monomorphic. In addition, males of passerine species otherwise classified as monomorphic often show slightly longer wings, longer tarsi, and/or a heavier body mass than females (Svensson, 1992). Measurement and subsequent discriminant analysis of these traits is a convenient way of morphologically distinguishing the sexes (Dechaume-Moncharmont et al., 2011). Here, we assess the validity of the beak shade criterion proposed by Buckley and Buckley (2004) and test for size dimorphism by comparing it to DNA sexing. In non-ratite birds, PCR product analysis of DNA taken from blood or feathers also yields distinct CHD gene patterns in

males and females (Griffiths et al., 1996; Griffiths et al., 1998; Zagalska-Neubauer and Dubiec, 2006; Ong and Vellayan, 2008).

We propose two new methods for sexing the Barbados bullfinch without the need of molecular techniques: i) first, we show that the lower mandible is darker in males, a feature that distinguishes the sexes with an 80% concordance with the PCR method, and ii) we show that a simple mathematical approach derived from discriminant analysis on morphometric traits distinguishes the sexes with a 97% concordance with the PCR method.

Methods

Fieldwork. Sixty-four Barbados bullfinches were captured in mist nets between February and April 2013 at eight different sites throughout the island of Barbados. Barbados bullfinches occur in almost all areas of Barbados islands (JNA, SD, and LL, personal observations), from highly urbanized areas to mostly rural habitats. We took advantage of a field study comparing birds from rural and urban populations to sample individuals in habitats with various urbanization rates (calculated using the percentage of anthropogenic structures in a 1 km^2 area around the capture point, Table 1). Beak shading (either pale or dark) was visually assessed by the same person (JNA) on all 64 birds at the moment of capture. Photographs were also taken at capture with a Nikon Coolpix P5100 camera for later re-assessment of shading judgments, with a gray scale image included in every picture. Post-hoc quantitative analysis of beak shade was performed by JNA, blind to the identity of the bird, measuring the optical density of the lower mandible divided by a standardized background of the gray scale, using the ImageJ v1.46r software (NIH, USA). Morphological measurements were taken at capture on all 64 birds by the same person (JNA); measurements were taken three times in succession on each bird and the mean value of the three measures was used in the analyses below. Individuals were weighed using a digital pocket scale (precision to 0.1 g). We measured tail length as the length of the longest straightened rectrix using a metal ruler (precision to 0.5 mm). Wing length was taken with a raised-end ruler as the length of the unflattened wing chord (precision to 0.5 mm). Calipers were used to measure the metatarsi, bill, and head (precision to 0.05 mm). Metatarsal length was measured from

the intertarsal joint to the last scale before the toes. Bill length was measured from the tip to the anterior edge of the nostril. Head length was measured from the anterior edge of the nostril to the back of the head following the angle of the bill. Measurement errors were calculated with the method proposed by Bailey and Byrnez (1990): $ME_{Wing\ length}$ = 4.3%, $ME_{Tail\ length}$ = 6.9%, $ME_{Tarsus\ length}$ = 9.5%, $ME_{Beak\ length}$ = 26.8%, $ME_{Head\ length}$ = 13.0%, indicating an overall mean measurement repeatability of 87.9%.

Birds were released at their initial site of capture. All manipulations were conducted according to Animal Use Protocol 2013-7140, approved by the McGill University Animal Care Committee and permit 8434/56 from the Natural Heritage Department of the Barbados Ministry of Environment and Drainage.

PCR sex-typing. After morphological measurements were taken, approximately 50 µL of blood was sampled by puncturing the brachial vein. Blood was kept at –20°C and shipped to the Jarvis laboratory at the Department of Neurobiology, Duke University Medical Center, Durham NC. DNA was extracted from the 50 µL of blood using a DNeasy tissue and blood extraction kit (Qiagen, USA). DNA quality and quantity was assessed on a Nanodrop (Thermo Scientific, USA). 50 ng of DNA was used for PCR. P8 (5′-CTCCCAAGGATGAGRAAYTG-3′) and P2 (5′-TCTGCATCGCTAAATCCTTT-3′) primers were used, following Griffiths et al. (1998). The PCR program was run as follows: 94°C for 1 min 30 s, 30 cycles of 48°C for 45 s, 72°C for 45 s and 94°C for 30 s, and then 48°C for 1 min and 72°C for 5 min (Griffiths et al. 1998). The PCR products were digested with HAEIII enzyme and then ran on a 1% agarose gel, as per Griffiths et al. (1996). Although P2 and P8 primers produce bands of slightly different molecular weights, we found that digesting with HAEIII facilitates differentiation and clearness of results (see Figure 2). Female samples display two bands (300 and 400 bp) whereas the male samples show only one band (300 bp). All molecular procedures were done by the same experimenter (JNA).

Statistical analyses. The correspondence between PCR sexing and our judgments on beak shade was tested using Fisher's exact test (Graphpad QuickCalcs, Graphpad software inc.). The correspondence between judgments on beak shade at capture and post-hoc optical density analyses was also assessed with a Fisher's exact test. For

morphological measurements, we first calculated mean differences between males and females identified by the PCR and tested them for significance with t-tests (Graphpad Prism 5, Graphpad software inc.). When data were not normally distributed, a Mann-Whitney test was used instead and when variances differed significantly, Welch's correction was applied. To validate our morphological model of sex differences, we built a discriminant analysis with traits that were significantly different between males and females (JPM 10.0, SAS Institute). To establish the model, we used a sample of 34 individuals randomly drawn from our 64 bird database and then applied the model to the other 30 birds to see how well it predicted PCR-determined sex.

Results

Of the 64 individuals we caught, all but one showed the brown feather coloration that is typical of both male and female Barbados bullfinches (Figure 1A). This is very different from coloration shown by the sister species of *L. barbadensis*, the Lesser Antillean bullfinch, in which the male is completely black with a red throat patch (Figure 1C). However, one of the individuals we caught in Barbados did exhibit a reddish throat patch (Figure 1B). PCR sexing identified 36 of our 64 birds as males (including the bird with the reddish patch) and 28 as females. Our judgments on the shade of the lower mandible (Figure 1D) yielded a high correspondence (80%) with the results of the PCR sexing: 51 of the 64 birds (Fisher's exact test $P < 0.0001$, 32 males and 19 females) had shadings that fit with the PCR result (Figure 2). Post-hoc optic density (OD) analysis of the mandible coloration yielded similar results (74% of birds correctly sexed by OD, Fisher's exact test $P = 0.0004$) with significant correspondence between both methods (Fisher's exact test $P = 0.0023$).

Morphology varied between the sexes on four of the six traits we quantified. Means were significantly different between PCR-sexed females and males for body weight ($\bar{x}_F = 16.3$, $\bar{x}_M = 17.3$, $P = 0.0006$), wing length ($\bar{x}_F = 66.4$, $\bar{x}_M = 70.6$, $P < 0.0001$), tail length ($\bar{x}_F = 48.3$, $\bar{x}_M = 51.5$, $P < 0.0001$) and beak length ($\bar{x}_F = 11.6$, $\bar{x}_M = 12.2$, $P = 0.0003$) (Table 2). Tarsus length and head length were not found to significantly vary between sexes.

The discriminant analysis model we built using the four significant morphometric differences was highly concordant with the PCR sexing. Quadratic discriminant analysis correctly predicted PCR sex 94% of the time (32 of 34 birds, $^{-2}$ log likelihood = 10.9, F = 11.13, $P < 0.0001$) on the model sample. When we applied this model to the other half of our database, PCR sex was correctly predicted in 29 out of 30 birds (97%, $^{-2}$ log likelihood = 8.89, F = 8.92, $P < 0.0001$).

Based on the discriminant analysis model, we derived a simplified formula that can be used to estimate sex using wing (W) and tail (T) length measurements: if (W*0.318 + T*0.796) ≥ 61.9, the bird is a male; if <, it is a female. It is as precise as the four-trait model yielded by the discriminant analysis, also predicting 29 of the 30 birds in the sub-sample not used to derive the model.

Discussion

Our results yield a fast, non-quantitative criterion for sexing the 'monomorphic' Barbados bullfinch in the field, with females showing a paler lower mandible than males. This result supports the suggestion made by Buckley and Buckley (2004) concerning the 'duskier' lower mandible of males. While this method is moderately accurate, it can be valuable for researchers wanting, for example, to perform a pre-selection of birds of a particular sex for a field study or manipulation, when no other methods are available. We also show that quantitative measures of two morphological traits, wing length and tail length, are sufficient to distinguish 97% of the males and females identified by the PCR analysis. Conveniently, these two traits are the easiest to measure accurately (our measurement repeatability scores were the highest for these two measurements). When field or captivity conditions preclude DNA sexing, our study thus offers a fast and easy solution to the identification of males and females in this species.

It has recently been argued that the use of morphometric traits to sex passerine birds is inaccurate when extrapolated to larger geographic scales (Ellrich et al., 2010). Here, we sampled sites with that vary sharply in urbanization rates, from less than 2% to more than 55%. A large part of Barbados is urbanized or used for agriculture, while limited forested areas remain in central and northern parts of the island. Contrary to previous studies on *L. barbadensis* parasitism and learning, which were all done on birds

caught in a limited urbanized coastal area in Saint James Parish, our eight sites were designed to cover both urban and rural habitats (Table 1). Our 64 individuals thus likely represent a rather wide sample for an endemic species restricted to Barbados island.

Phylogenetic studies of *Loxigilla noctis* and *L. barbadensis* in the Lesser Antilles (Lovette et al., 1999) suggest that the Barbados bullfinch is a relatively recent immigrant to that island and that a single invasion event is behind the low within-population nucleotide divergence of the species there. The most parsimonious scenario for the lack of color dimorphism in the Barbados bullfinch is thus that the trait has been lost with respect to its common ancestor with *L. noctis.* Our anecdotal finding that a version of the *L. noctis* red throat patch can still be found, albeit in only one of 36 PCR sexed males, on a background of brown body coloration, supports the idea of trait loss in *L. barbadensis*. Although we cannot exclude the possibility that this bird could be a migrant from other islands, it is unlikely since the overall plumage coloration is much closer to *barbadensis* than *noctis* and, to our knowledge, no brown male has been reported in other Caribbean islands. The genetic basis of this loss would be interesting to explore, as would be the effects of female preference on Barbados males painted with the *L. noctis* colors or the reddish throat patch found in our single *L. barbadensis*.

The reason for the male shift to female coloration in Barbados is unknown. Intriguingly, Carib grackles (*Quiscalus lugubris*) have also evolved a monomorphic plumage coloration in Barbados, but in the opposite direction to bullfinches: it is female grackles that have changed their coloration, from the brown seen on other islands of the Lesser Antilles, as well as northern South America, to the black plumage characteristic of all *Q. lugubris* males. Overington (2011) has suggested that the low levels of predation in Barbados may have favored this shift towards monochromatism. Predation rate is known to affect bird coloration, with a higher predation rate being correlated with plumage dullness (Martin and Badyaev, 1996). The Barbados population of Carib grackles is thought to have originated in northern South America (Lovette et al., 1999) and predation there is much higher than it is in Barbados. Venezuela, for instance, has several native Carnivora and 65 species of diurnal raptors (Naveda-Rodriguez, 2013), whereas there are only rare vagrant predators in Barbados (e.g., Peregrine falcon, *Falco*

peregrinus). Contrary to grackles, however, Barbados bullfinches are thought to have originated from the small neighboring island of Saint-Lucia (Lovette et al., 1999), where predators are also very scarce. Therefore, predation rate is unlikely to have affected sexual dichromatism in Barbados bullfinches.

In birds, species tend to be monochromatic when both sexes participate in parental duties, including nest building (Verner and Willson, 1969, Kear, 1970, Soler et al., 1998). Bird (1983) has shown that male Barbados bullfinches, compared to male Lesser Antillean bullfinches, contribute more to nest building, stay longer in the vicinity of their nest after construction and throughout brood rearing, feed females more often and are more aggressive around their nest (Bird, 1983, Buckley and Buckley, 2004). Breeding system might thus be an important factor in the loss of male dimorphism in this species.

Whatever the reason for the loss of dichromatism, our study validates two rapid methods for the sexing of *L. barbadensis* that can be used in the absence of invasive techniques, and raises fundamental questions with regards to the evolution of sexual dimorphism.

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Figures

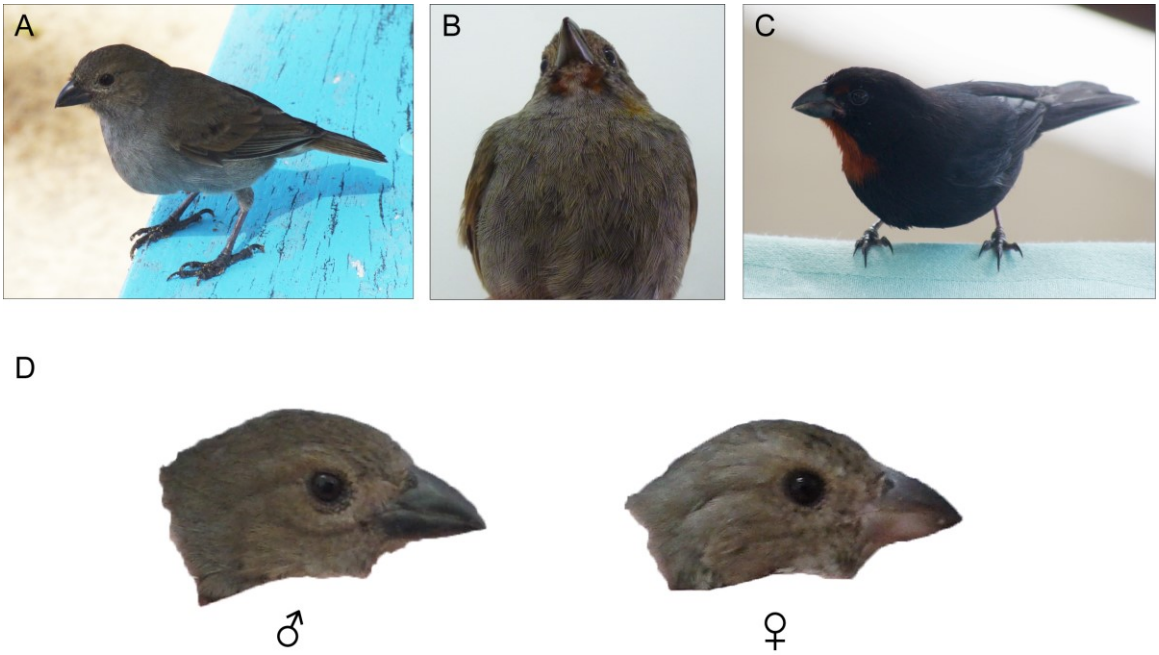

Figure 1. Sexual dimorphism in Barbados bullfinches and Lesser-Antillean bullfinches. (A) Male B. bullfinch showing no obvious sexual dimorphism. (B) Male B. bullfinch showing a reddish throat patch. (C) Lesser Antillean Bullfinch (*Loxigilla noctis sclateri*) displaying strong sexual dimorphism. (D) Lower mandible shade of a male versus a female Barbados bullfinch.

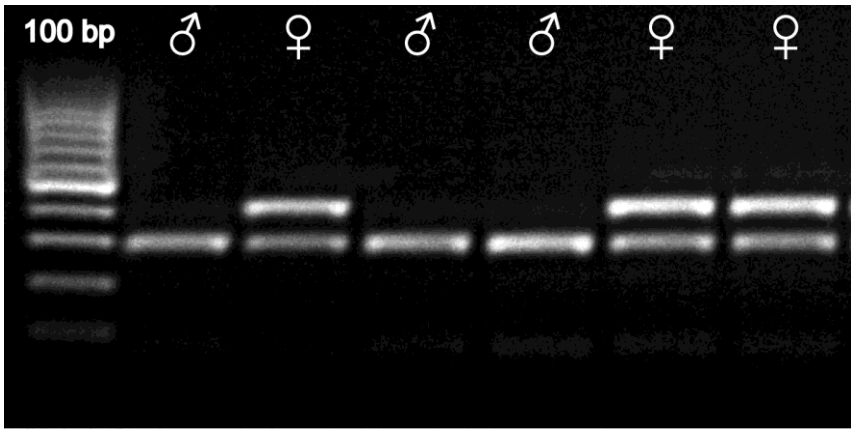

Figure 2. Sex-typing PCR. Representative samples analyzed for sex determination. After agarose gel migration of the PCR products, there is one band (~400 bp) for males and two bands (~400 and ~500 bp) for females.

Tables

Table 1. Summary of captured bullfinches and their site of capture

Site name	GPS coordinates	Anthropization	n
Bellairs	+13° 11' 31.21" , -59° 38' 25.20"	18.0%	12
Bridgetown	+13° 5' 50.98" , -59° 37' 21.65"	54.7%	10
Bruce Vale	+13° 13' 18.98" , -59° 33' 30.74"	2.4%	9
Jamestown park	+13° 11' 18.84" , -59° 38' 7.79"	21.1%	9
Swans	+13° 14' 10.96" , -59° 35' 17.16"	2.1%	7
White Hill	+13° 13' 18.24" , -59° 34' 31.68"	3.7%	7
Jah	+13° 15' 18.80" , -59° 35' 14.56"	5.6%	5
Payne's Bay	+13° 9' 47.83" , -59° 38' 10.71"	25.3%	5
Total			**64**

Summary of all captured bullfinches by site. Anthropization was measured as the percentage of a satellite map covered by human landmarks (roads, buildings, etc.)

Table 2. Morphometric sex differences in Barbados bullfinch

	Sex	*n*	Mean	Min	Max	SEM	95% conf. Interval	Cohen's *d*	t-test *p*
Body weight (g)	**F**	28	**16.3**	14.0	18.7	0.25	15.75 - 16.76	**0.7**	**0.0055**
	M	36	**17.2**	14.8	22.2	0.22	16.81 - 17.68		
Wing length (mm)	**F**	28	**66.4**	63.5	69.5	0.32	65.76 - 67.07	**1.7**	**< 0.0001**
	M	36	**70.6**	63.0	81.0	0.56	69.42 - 71.69		
Tail length (mm)	**F**	28	**48.3**	43.5	51.0	0.30	47.68 - 48.93	**2.2**	**< 0.0001**
	M	36	**51.5**	48.5	55.0	0.23	51.05 - 51.97		
Tarsus length (mm)	**F**	28	23.0	21.1	24.2	0.12	22.73 - 23.22	0.3	0.1866
	M	36	23.2	21.5	24.4	0.12	22.97 - 23.44		
Beak length (mm)	**F**	28	**11.6**	10.6	12.8	0.10	11.43 - 11.82	**1.0**	**0.0003**
	M	36	**12.2**	11.0	13.5	0.11	11.99 - 12.44		
Head length (mm)	**F**	28	17.6	16.7	19.0	0.10	17.40 - 17.80	0.5	0.0962
	M	36	17.9	16.3	19.3	0.13	17.61 - 18.14		

Means and SEM for morphological measurements of PCR-sexed males and females. P-values were computed using unpaired bilateral Student's t-tests. A Welch's correction was applied for head length to correct for different variances and a non-parametric t-test was used for wing length to account for the non-Gaussian distribution.

Appendix II.

What's flexible in behavioral flexibility?

Audet J.-N. and Lefebvre L.

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Abstract

Behavioral ecologists interested in comparative cognition have struggled to design tasks that are both ecologically relevant and experimentally rigorous. In experimental psychology, standardized tests of reversal learning, set-shifting and self-control have long been used to measure aspects of flexible behavior especially with regards to determining the neural mechanisms that enable animals and humans to rapidly and efficiently adapt to different situations. More recently, behavioral ecologists have used the term "behavioral flexibility" more broadly to explain differences in traits such as personality and innovation. Here, we argue that the term behavioral flexibility designates too many non-equivalent traits, and that this can lead to misconceptions about the nature of cognitive abilities.

Introduction

The terms "behavioral flexibility" and "cognitive flexibility", used interchangeably, have been employed for decades in the field of experimental psychology to label a form of cognition that enables animals and humans to adapt their behavior to changing environmental contingencies (Grattan & Eslinger 1989; Ragozzino et al. 1999; Floresco et al. 2009). In experimental psychology, commonly used tests of behavioral flexibility include reversal learning, set-shifting and self-control. In recent years, the term has featured prominently in behavioral ecology, where it is sometimes applied in the same manner as in psychology via tests of reversal learning, but more often in the context of innovation (Sol et al. 2002; Reader & Laland 2003) and problem-solving (Leal & Powell 2012). However, there is increasing evidence that innovative problem-solving and reversal learning are distinct, if not opposite, abilities (Griffin et al. 2013), while within psychology, different tests of flexibility may well be measuring different traits (Griffin & Guez 2014). If we add to this the many unrelated phenomena that the term has been applied to (e.g. animal personality: van Overveld & Matthysen 2013; defense mechanisms: Stoekl et al. 2015; division of labor: Kwapich & Tschinkel 2016), there is a clear risk that behavioral flexibility as a concept, let alone a term, will completely lose its significance.

Here, we briefly survey the ways in which behavioral flexibility has been assessed and conclude that even if the different assays used in behavioral ecology are conceptually linked, there is little empirical evidence that they are related. We argue that referring to such a large number of potentially non-equivalent and non-related skills with a single term is not useful, often misleading and should be avoided.

Behavioral flexibility in psychology

In experimental psychology, the concept of behavioral flexibility emerges from principles of animal learning (Sutherland & Mackintosh 1971; Dickinson 1981) in which an animal makes a decision or choice that is largely influenced by various schedules of reward and future reward outcomes (Clarke et al. 2004; Chudasama 2011). One commonly used scenario, and one that has been readily adopted in comparative studies, is the *reversal learning* paradigm, where a dominant response must be

overridden due to changes in reward contingencies (Jones & Mishkin 1972; Rolls 2000). First, the animal associates one rewarded conditional stimulus (CS+) with a response leading to a reward in the presence of a second, unrewarded stimulus (CS-). This process may continue over several hundred trials, encouraging the formation of a dominant response. Then, unknown to the animal, the stimulus-reward contingency is reversed and the animal must now change its response and use the previously unrewarded stimulus as a cue. There is some response persistence to the initially rewarded stimulus, as would be expected, before the animal works out the new rule. In some cases, however, the response persistence may be exaggerated. This is the case, for example, of rats or monkeys (marmosets and macaques) with orbitofrontal damage, indicating that this structure is involved in reversal performance (Dias et al. 1996; Schoenbaum et al. 2003; Chudasama & Robbins 2003; Izquierdo et al. 2004; Kim & Ragozzino 2005; Jang et al. 2015).

Related to reversal learning is the set-shifting paradigm, where the animal's attention is solicited by different stimulus dimensions and the animal must alternate between strategies, rules, and attentional sets (Roberts et al. 1988). The cues can be olfactory, tactile, visual and spatial at the same time. The subject needs to first focus on one type of stimulus (for example, a rewarded and an unrewarded stimulus that differ in color) to get the reward as in a classic discrimination learning task, but then it must switch to another stimulus dimension (for example, spatial position or shape or texture) to distinguish the rewarded and unrewarded stimuli in the next phase, ignoring the previously rewarded color dimension (Dias et al. 1996; Oswald et al. 2001; McAlonan & Brown 2003; Brigman et al. 2005). Therefore, in set-shifting, the rule is less tangible and the animal must form multi-dimensional attentional sets and shift between them to succeed. Although reversal learning and set shifting are related, they are anatomically dissociable: reversal learning, which involves adapting behavior in accordance with changes in stimulus-reward contingencies, requires an intact orbital prefrontal cortex in mammals, whereas switching attention between perceptual dimensions as in set-shifting relies on the lateral prefrontal cortex in primates or medial prefrontal cortex in rats (Chudasama & Robbins 2006; Nilsson et al. 2015). In sum, set-shifting tasks are designed to measure the subject’s ability to switch strategies, rather than simply learn a

new association by reversing a previous one, and this is reflected in the different neural circuits that are involved in the two tasks.

Self-control is considered to be another aspect of behavioral flexibility both by experimental psychologists (see review by Coutlee & Huettel 2012) and behavioral ecologists (Amici et al. 2008; Boogert et al. 2011). Sometimes also referred to as "cognitive control", self-control is defined as the extent to which an animal is able to withhold or inhibit its action in the face of a more immediate apparent reward. One way of testing for spatial self-control is the detour-reaching task, commonly used in comparative studies, which requires the animal to inhibit direct attempts to reach a visible food reward in a transparent apparatus, and to instead make a detour around the transparent obstacle to retrieve the food (Diamond 1990). Self-control probably involves different brain areas than do reversal learning and set-shifting, at least in humans (Aron et al. 2014). Although this is still a matter of debate, the right inferior frontal cortex seems to be one of the main areas responsible for self-control (Aron et al. 2014; Swick et al. 2008). In short, self-control tasks assess a subject's ability to inhibit its initial response of using the simplest route or strategy to focus on an indirect, but more efficient approach, an ability that appears to be neurologically distinct from reversal and set-shifting tasks' proficiencies.

Behavioral ecologists that look to psychology for standardized, well documented assays of animal cognition should thus be aware that, whatever the conceptual similarities between the tasks described above, there are thus clear differences in the traits that they measure (reversal of an association, attention to different cue dimensions, inhibition or impulse control), as well as their neural substrates (e.g., lateral prefrontal, orbitofrontal or right inferior frontal cortex; Wallis et al. 2001; Chudasama & Robbins 2003; Chudasama et al. 2003; Rudebeck et al. 2006; Kuhn et al. 2009; Sharp et al. 2010; Aron et al. 2014). This heterogeneity needs to be taken into account when transposing tasks and their interpretation to the more naturalistic situations that behavioral ecologists usually focus on. For example, although detour-reaching and reversal learning are both said to measure flexibility, a study on wild-caught song sparrows found that the two tasks had *opposite* relationships with song repertoire: repertoire size had a positive

relationship with detour reaching performance, but a negative one with reversal learning (Boogert et al. 2011).

Behavioral flexibility in ecology

Behavioral ecologists sometimes use the same tasks as experimental psychologists (Bond et al. 2007), but they also often think of behavioral flexibility in terms of innovation and problem-solving (Reader & Laland 2002; Sol et al. 2002; Tebbich et al. 2010; Wright et al. 2010; Huebner & Fichtel 2015). Innovation is defined in non-humans as a solution to a novel problem or a novel solution to an old problem (Kummer & Goodall 1985). Extractive foraging problems requiring obstacle removal have become a classic experimental test for innovation, following decades of studies on the origin and spread of the oldest (1921) animal innovation in the literature, the opening of milk bottles by tits (Fisher & Hinde 1949). While there is still a debate about the relative roles of persistence, motor diversity and cognition in the solving process (Griffin et al. 2014; Quinn et al. 2014; Rowe & Healy 2014; Thornton et al. 2014; Morand-Ferron et al. 2015; Pritchard et al. 2016; Diquelou et al. 2016; Cauchoix & Chaine 2016), there is some agreement that obstacle removal problems are a good way of assessing innovative foraging in experimental tests (Griffin & Guez 2014). Studies on birds are the most numerous in this field (Thornton & Samson 2012; Benson-Amram et al. 2016). Overall, the studies suggest a negative (interindividual: Griffin et al. 2013; Tebbich & Teschke 2014, interspecific: Tebbich et al. 2010; Tebbich et al. 2012) or zero (interindividual: Boogert et al. 2011; Isden et al. 2013; Shaw et al. 2015; Logan 2016, interpopulational: Audet et al. 2016) relation between reversal learning and problem-solving performance**.** Likewise, problem-solving and detour reaching performance are often uncorrelated in birds (Boogert et al. 2011), but also interspecifically in great apes: orangutans are by far the best of the four great apes in detour-reaching (Vlamings et al. 2010), but the worst in an extractive problem requiring repeated innovation (Manrique et al. 2013).

By their very nature, reversal learning tasks might measure very different processes than the ones measured by innovation and extractive foraging problems. In a reversal task, there is a sudden change in the relationship between two cues and a reward, such that the cue that repeatedly predicted the reward in preceding trials is no longer

predictive, and the cue that never predicted reward becomes highly predictive. In serial reversals, previously correct cues repeatedly become suddenly incorrect and previously incorrect cues repeatedly become correct. Persistence leads to errors in such tasks (Nilsson et al. 2015), but persistence is on the contrary a strong facilitator of success in innovative problem-solving (Gajdon et al. 2006; Tebbich et al. 2010; Overington et al. 2011; Thornton & Samson 2012; Cole & Quinn 2012; Benson-Amram & Holekamp 2012; Cauchard et al. 2013; Huebner & Fichtel 2015; Griffin & Guez 2016). Chow et al. (2016) have recently tested the effects of persistence and flexibility on problem-solving efficiency in grey squirrels. They found that "flexibility", measured as the rate of switching between tactics, was not linked to problem-solving performance, whereas persistence was a strong predictor of success. In addition, the sudden and often repeated changes in cue value in reversal and set-shifting tasks characterize neither extractive foraging problems in captivity or innovation cases in the wild. In fact, the problems that are solved in the wild are often very similar to captive extractive foraging problem-solving tasks but, to our knowledge, do not resemble reversal learning tasks.

While neural substrates of innovative problem-solving are still poorly understood, a few studies on laboratory rodents points to specific areas of the prefrontal cortex. In mice, inactivation of the medial prefrontal cortex causes deficits in an obstacle removal problem (Ben Abdallah et al. 2011) and in set-shifting, but not in reversal learning (Floresco et al. 2008), while in rats, the beta-adrenergic antagonist propranolol negatively impacts obstacle removal but not set-shifting (Hecht et al. 2014). This neurobiological evidence, together with correlational data, suggests that innovative problem-solving and other behavioral flexibility measurements are distinct proficiencies. As most recent studies of innovative problem-solving are done on birds, it should be noted that multiple lines of evidence point to the nidopallium caudolaterale (NCL) as the equivalent of the mammalian prefrontal cortex, providing a clear candidate structure for the control of similar behaviors in the two classes (Mogensen & Divac 1993; Rose & Colombo 2005; Rose et al. 2010; Herold et al. 2011; Helduser & Güntürkün 2012; Shanahan et al. 2013; Veit & Nieder 2013; Lengersdorf et al. 2015).

Innovating in the flexible usage of the term

While reversal learning, innovation and problem-solving dominate the literature on flexibility in behavioral ecology, the term has recently come to be used to qualify a surprisingly broad range of behaviors, including variation in neophilia/neophobia in primates (Bergman & Kitchen 2009), exploratory behavior in birds (van Overveld & Matthysen 2013), vigilance level in birds (Couchoux & Cresswell 2012), tool-use in primates (Vale et al. 2016), nest site choice in turtles (Barsante Santos et al. 2016), division of labor among colony members in ants (Bernadou et al. 2015; Kwapich & Tschinkel 2016) or between parents in frogs (Ringler et al. 2015), daily activity allocation in fish (Fingerle et al. 2016), niche allocation in rats, fish and birds (Igulu et al. 2013; Hunt 2016; Loveridge et al. 2016), courtship timing in spiders (Bardier et al. 2015), adjustment of feeder use in birds (Herborn et al. 2014), social organization in primates (Otani et al. 2014; Kamilar & Baden 2014), trial-and-error (discrimination, not reversal) learning in bats (Zhang et al. 2014), diversity of material used for nests in bees (MacIvor & Moore 2013), intensity of chemical defense in wasps (Stoekl et al. 2015), foraging activity across trials in fish (Adriaenssens & Johnsson 2011), degree of soft tissue retraction in foraging snails (Edgell et al. 2009), and the adjustable choice of suction or compression to process food items in elasmobranches (Wilga et al. 2012). This rich diversity of behavioral investigations is useful, as it provides a detailed picture of how behaviors are modified under changing conditions. However, there is little chance that all these cases share a similar etiology. Therefore, referring to this huge diversity of traits under the same blanket term is problematic. Within certain limits, a concept can be multifaceted, but the number and nature of the different contexts in which 'behavioral flexibility' has been applied seems excessive. Based on the actual flexible usage of the term, flexibility is attributed to such a wide array of behaviors that are likely to have very different underpinnings that the term is more confusing than useful, especially in cognitive ecology. In this field, experiments implicitly or explicitly aim to understand the cognitive, and eventually neural processes, behind the behaviors tested. Given the mechanistic implications of such experimental studies, the use of a blanket term is especially problematic, as studies within both psychology and behavioral ecology point to heterogeneity in the co-variation and neural underpinnings of the

different assays. In large scale comparative analyses of innovation in the wild (Reader & Laland 2002; Sol et al. 2002), where the focus is not on mechanisms, but on a wide variety of manifestations that go from simple incorporation of new foods in the diet to more sophisticated technical skills (Overington et al. 2009; Ducatez et al. 2015; Navarrete et al. 2016), the problem is less acute, but still preoccupying.

Conclusion

What's flexible in behavioral flexibility? In brief, ways of measuring it. Our review suggests that different assays of behavioral flexibility used in experimental psychology and behavioral ecology are not necessarily equivalent, do not co-vary and are controlled by different neural mechanisms. Some of these assays are even designed to assess opposite abilities, given the contrasting effect of persistence on the performance of each task. Consequently, referring to innovative problem-solving, reversal learning, set-shifting, self-control or other even more distant traits under the same general term of behavioral flexibility can lead to misconceptions about how behavior should be interpreted, especially when comparing cognitive mechanisms across species. Thus, we suggest that the term should be avoided, at least in behavioral ecology. The precise tasks used to assess flexibility in experimental studies, whether they use the standard tasks and model species of psychology or the more naturalistic context of behavioral ecology, should be specified, as we gain more and more detailed knowledge of the mechanisms and the neural events that regulate the different ways in which animals change their behavior in the face of environmental challenges.

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Appendix III.

Feeding innovations in a nested phylogeny of Neotropical passerines

Lefebvre L., Ducatez S. and Audet J.-N

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Abstract

Several studies on cognition, molecular phylogenetics and taxonomic diversity independently suggest that Darwin's finches are part of a larger clade of speciose, flexible birds, the family *Thraupidae*, a member of the New-World nine-primaried oscine superfamily *Emberizoidea*. Here, we first present a new, previously unpublished, data set of feeding innovations covering the Neotropical region and compare the stem clades of Darwin's finches to other neotropical clades at the level of the subfamily, family and superfamily/order. Both in terms of raw frequency as well as rates corrected for research effort and phylogeny, the family *Thraupidae* and superfamily *Emberizoidea* show high levels of innovation, supporting the idea that adaptive radiations are favoured when the ancestral stem species were flexible. Second, we discuss examples of innovation and problem solving in two opportunistic and tame Emberizoid species, the Barbados bullfinch *Loxigilla barbadensis* and the Carib grackle *Quiscalus lugubris fortirostris* in Barbados. We review studies on these two species and argue that a comparison of *L. barbadensis* with its closest, but very shy and conservative local relative, the black-faced grassquit *Tiaris bicolor*, might provide key insights into the evolutionary divergence of cognition

A nested phylogeny of flexible New World birds

The superfamily *Emberizoidea*, also known as New World nine-primaried oscines (Barker et al. 2013), includes the families *Emberizidae, Icteridae, Parulidae,* and *Cardinalidae*, as well as *Thraupidae*, whose most famous members are Darwin's finches. The superfamily accounts for almost 8% of all birds (832 species, Barker et al. 2015) and has evolved a broad range of morphologies and feeding adaptations that have allowed it to radiate throughout the New World, parts of the Old World (buntings) and to colonise outlying islands in the Pacific (Galápagos finches, Cocos finch) and Atlantic oceans (Tristan da Cunha finches, Gough finch) (Ryan et al. 2013). The diversification rate of the superfamily, based on statistical comparisons (Ricklefs 2003) and molecular estimates of divergence time from common ancestors (Barker et al. 2013) is higher than that of other clades, with the families *Icteridae* (grackles, cowbirds and New World blackbirds) and *Thraupidae* (collectively referred to as tanagers) contributing most of the effect.

The family *Thraupidae* in particular has a 40% higher diversification rate than its most closely related clades, five times higher than that of the *Neoaves* mean and an order of magnitude higher than the vertebrate average (Barker et al. 2013). Recent revisions of *Thraupidae* molecular phylogeny (Burns et al. 2014) have led to the incorporation into this family of many species previously classified (Jetz et al. 2012) as *Emberizidae*. This includes Darwin's finches, as well as several Caribbean bullfinch and grassquit genera, plus the bananaquit *Coereba flaveola* that had earlier been considered the sole member of the *Coeribidae*. This revision makes tanagers the second largest family of birds, representing 12% of the Neotropical avifauna (371 species, Burns et al. 2014).

Within *Thraupidae*, the subfamily *Coerebinae*, to which Darwin's finches belong, shows a range of trait variation (for example, bill dimensions) that is much higher than that of other subfamilies with similar ages and levels of sequence divergence (Burns et al. 2002). Because of this range of trait variation, the high diversification rate, and the ability to disperse from South and Central America to islands in the Caribbean as well as the Pacific and Atlantic oceans, Burns and co-authors (2014) go as far as

suggesting that the *Coeribinae* might have intrinsic evolvability, i.e. a greater propensity for dispersal than other lineages, a greater capability of colonising islands and a developmental-genetic architecture that includes a greater variety of regulatory genes leading to a higher degree of phenotypic variation in key traits (see also Burns et al. 2002; Mallarino et al. 2012). For example, different lineages of Darwin's finches and endemic Caribbean bullfinches show both variation and convergence in the genetic system guiding the bone and cartilage development that determines beak size and shape (Mallarino et al. 2011; Mallarino et al. 2012). Chaves and co-authors (2012) contrast the large morphological variation seen in Darwin's finches with the lack of variation observed in the yellow warblers that have also colonised the Galápagos and Cocos islands; like Burns et al. (2002; 2014), they also raise the possibility of differences in evolvability between the clades.

Independently of this literature on molecular phylogenetics and developmental genetics, Tebbich et al. (Tebbich et al. 2010) applied West-Eberhard's (2003) concept of 'the flexible stem' in discussing both the speciosity and cognitive abilities of Darwin's finches. In her book, West-Eberhard (2003) had proposed that adaptive radiations may be favoured when an exceptionally flexible stem species colonises a new environment. In comparing the tool-using woodpecker finch *Camarhynchus pallidus* and its non tool-using sister species, the small tree finch *Camarhynchus parvulus*, Tebbich and colleagues (2010) found no evidence that the former had an adaptively specialized form of physical cognition that differed from its non-tool using relative. Tebbich and co-authors (2010) proposed that innovativeness might be phylogenetically primitive in the clade and that flexibility within the founding population of the Galápagos had led to the development of new behaviours to exploit the new foods and new habitats the colonisers found there. Given genetic variation, selection had then, over time, led to several cases of genetic accommodation.

What is striking about this 'flexible stem hypothesis' is its similarity to the conclusions arrived at by the analysis of molecular diversification and phenotypic variation: the highly innovative, tool using woodpecker finch shares key traits with the whole, speciose, clade of Darwin's finches, who share these traits with their relatives in

the whole *Coeribinae* subfamily, the whole tanager family and several branches of the *Emberizoidea* superfamily. In other words, high innovativeness, high phenotypic variation and high diversification rates might be shared traits of a nested phylogeny that goes from the species to the superfamily. The 'flexible stem' might thus be ancient.

Our paper addresses this possibility in two ways, combining a phylogenetic analysis of a new, previously unpublished, data set of innovations from the Neotropical region and a discussion of innovations and problem solving in two well-studied Emberizoid species from Barbados. The new Neotropical innovation database is given in its entirety in Supplementary Table 1. If the flexible stem hypothesis applies to Darwin's finches, we predict that the nested clades (subfamily *Coeribinae*, family *Thraupidae*, superfamily *Emberizoidea)* that lead to Darwin's finches should show high innovation frequencies. To do this, we draw on the same method used for previous innovation databases (birds: North America and the British Isles: Lefebvre et al. 1997; Australia and New Zealand: Lefebvre et al. 1998; Western Europe and the Indian subcontinent: Timmermans et al. 2000; primates: Reader and Laland 2002), an exhaustive search of the short notes of as many local specialized journals as we could consult. The second part of our paper reviews field and experimental data on innovativeness in one of the Darwin's finches closest relatives, the endemic Barbados bullfinch *Loxigilla barbadensis*. We also extend our discussion of field and experimental data to the most innovative genus within *Emberizoidea*, the grackle genus Quiscalus, in particular the highly opportunistic species that feeds with *L. barbadensis* in the wild, the Carib grackle *Q. lugubris fortirostris.*

Comparative analyses of feeding innovations in Neotropical birds

We exhaustively searched the short notes of all Neotropical ornithology journals available to us online at McGill (37 journals from Mexico to Chile; see Supplement 1 for details of the methods) for key words mentioning opportunism (112 cases; note that a given case may contain several key words), 'not' or 'never' or 'un-' recorded behaviours (111 cases), 'first' reports (56 cases), 'new' and 'novel' (44 cases) or 'unusual '(9 cases) observations that 'depart' from the usual behaviour (30 cases) or have

been seen 'only' in 'other' species or 'other' foods (42 cases) or are 'learned' (5 cases). As in previous databases, we used the judgment of the author of the primary observation as a criterion for inclusion, as Neotropical ornithologists know their study species better than we do.

We found 352 innovations in 256 species. The entire database is given in Supplementary Table 1. Innovations ranged from simple opportunistic feeding on a newly available food source (often insects) to the more spectacular cases of an Antarctic skua (*Stercorarius antarcticus)* and a blackish cinclodes *(Cinclodes antarcticus*) drinking blood from a wound on an elephant seal, tool use in the shiny cowbird (*Molothrus bonariensis)* and the yellow-rumped marshbird *(Pseudoleistes guirahuro)* and baiting fish with bread in the rufescent tiger heron (*Tigrisoma lineatum)*, a behaviour normally reported in the striated heron (*Butorides striata)* and other heron species (see review in Ruxton and Hansell 2011).

Figures 1 to 3 present phylogenetic diagrams of innovation rate per clade at three taxonomic levels: *Emberizoidea* against other superfamilies and orders (figure 1), *Thraupidae* against other nine-primaried oscine families (figure 2) and *Coeribinae* against other Thraupid subfamilies (figure 3). In these diagrams, taxa are placed according to their phylogenetic proximity and innovation rates (right part of each figure) are calculated as residuals of Phylogenetic Generalized Least Squares (PGLS) regressions of innovation frequency (left part of each figure) against research effort (taken from Ducatez and Lefebvre 2014) per clade, which is an important confounding variable of innovation frequency ($p = 0.058$ to 0.0003 in this dataset depending on the taxonomic level; see Supplement 1). Clades where no innovations were found are not included in the analyses, as the absence of innovations might mean either that birds of these clades are not innovative or that whatever innovations they might show were not observable for geographic or research effort reasons (research effort on 2217 of avian species worldwide is zero, Ducatez and Lefebvre 2014). The phylogenetic signal was high at the superfamily and subfamily levels (Pagel λ estimated by maximum likelihood = 0.934 and 1), but null at the family level (Pagel $\lambda = 0$), suggesting that variation in innovativeness between families is independent of phylogeny.

As is evident in the left part of each figure, the nested phylogeny that goes from *Emberizoidea* to *Coeribinae* reveals high innovation frequencies at all three taxonomic levels. When frequencies are regressed against research effort and common ancestry controlled in the PGLS, however, only the higher two phylogenetic levels, the superfamily and the family, reveal high innovation rates for the nested clades that include Darwin's finches. At the highest taxonomic level (figure 1), *Emberizoidea* have the largest number of innovations (71, figure 1a), as well as positive phylogenetically-corrected residuals (figure 1b) that are only slightly smaller than those of the two suboscine infraorders *Tyrannida* (tyrant flycatchers) and *Furnarida* (ovenbirds*)*. As in other parts of the world (Overington et al. 2009), *Piciformes* (in the neotropics, toucans as well as woodpeckers), gulls (suborder *Lari*) and raptors (*Falconiformes* and *Accipitriformes*) show high innovation frequencies (figure 1a). Caracaras are the species group with the highest number of innovations, 18, the genus *Milvago* (8 innovations) and *Caracara plancus* (7 innovations) providing the largest share (see Supplementary Table 1). As is the case in other innovation databases (Overington et al. 2009), shorebirds (suborders *Scolopaci* and *Charadrii*) and doves (*Columbiformes*) show low innovation frequencies. Ratites and *Galloanserae* also show either zero or very low innovation rates: ducks and landfowl are absent from figure 1 because they show no innovations, while the greater rhea registers the only known ratite innovation worldwide, with the presence of fish in faeces supporting an observation of consumption of fish at the margins of a reservoir (Azevedo et al. 2006).

Several passerine clades show high innovation rates, in particular the suboscine infraorders *Tyrannida* and *Furnarida*. Surprisingly, corvids (*Corvoidea)* do not dominate the database the way they do in all other parts of the world (Overington et al. 2009) and rank 12th and 9th respectively in terms of innovation frequency and phylogenetically-corrected residual rate in the Neotropics. The two dietary categories that are the source of many innovations in other parts of the world, predation and carrion feeding, seem to be rare in South American Corvids (Lopes et al. 2005). Instead, Lopes et al. (2005) highlight the fact that two Thraupid species show corvid-like ingestion of meat remains on cattle skin drying in the sun.

At the level of families within nine-primaried oscines, *Thraupidae* rank first with 56% of the innovations in the clade (41 of 71; figure 2a). *Icteridae* (grackles, cowbirds and allies) rank second on both the left and right part of figure 2. Within *Thraupidae*, the subfamily *Coeribinae* ranks highest in terms of innovation frequency (16, figure 3a), but falls behind other subfamilies when research effort, elevated by the many studies on Darwin's finches, is factored in with the PGLS (figure 3b). As in other innovation data bases (Lefebvre et al. 1997; Lefebvre et al. 1998; Timmermans et al. 2000; Overington et al. 2009), our focus on low impact factor regional ornithology journals might have underestimated innovation rates in taxa where the most spectacular cases are reported in higher impact factor journals, which are included in research effort, but not in innovation frequency. We are currently estimating the effects of this limitation on our worldwide database. Due to this possible limitation, the family level provides more robust support of the flexible stem hypothesis than the subfamily level.

A review of innovativeness and problem solving in *Loxigilla barbadensis* and *Quiscalus lugubris fortirostris*

The neotropical innovation database clearly supports the flexible stem hypothesis at all three taxonomic levels when innovativeness is measured as uncorrected frequencies, and at the levels of the family and superfamily when innovation frequencies are corrected for research effort and phylogenetic signal**.** Beyond the comparison of innovation rates in the wild, however, a more complete understanding of innovativeness requires experimental assays that can be transferred to captivity. Problem solving tasks, especially those that involve the removal of obstacles blocking access to food, have proven useful for this (Griffin and Guez 2014). It was both Darwin's finch innovativeness in the wild and their strong performance in problem-solving tasks (Teschke et al. 2011; Teschke and Tebbich 2011; Tebbich et al. 2012; Teschke et al. 2013; Tebbich and Teschke 2014) that led Tebbich and co-workers to apply the flexible stem hypothesis to this clade (Tebbich et al. 2010). If our innovation data suggest that the stem is at the level of the family and superfamily, we should be able to identify other

innovative *Thraupidae* and New World nine-primaried oscines that also show enhanced problem-solving abilities.

The island of Barbados hosts two Emberizoid species that are good candidates, the endemic bullfinch *Loxigilla barbadensis*, and the Carib grackle *Quiscalus lugubris fortirostris*. Both species are dietary generalists. Barbados shares many of the features that facilitate innovative behaviour in finches of the Galápagos: tameness due to a historically low level of predation, wide niches due to low levels of competition from a paucity of avian species, and limited resources due to small island size. Barbados lacks the dryness extremes that make the Galápagos a particularly challenging environment, but it has an additional feature that favours behavioural plasticity, intense anthropogenic modification of the original environment, providing birds with many novel habitats and food sources as a result of urbanisation and agriculture.

Several studies in the field and in captivity have documented the opportunism, innovativeness and problem-solving abilities of *L. barbadensis* and *Q. lugubris fortirostris.* We briefly review them here. In the field, Carib grackles take dry food pellets from dog bowls and soften them by dipping them in water (Morand-Ferron et al. 2004; figure 4a]. Some individuals steal the dunked pellets when they are dropped in water by a conspecific (figure 4b), and the frequency of dunking is determined by social (flock size, theft) and energetic (distance to water, consumption time of dunked versus dry food) costs and benefits (Morand-Ferron et al. 2004; Morand-Ferron et al. 2006; Morand-Ferron et al. 2007). The relationship between dunking and stealing follows the frequency-dependent payoffs of a producer-scrounger game (Morand-Ferron et al. 2007). Barbados grackles have been seen foraging for dead insects under the windshield wipers of parked cars, as well as passing bread and rice to a begging juvenile through the wire mesh of its cage during captive experiments (S. M. Reader et al. 2002). Grackles were also observed several times eating fish remains at the Payne's Bay fish market (St-James; figure 4c). This behaviour is typical of cattle egrets in Barbados (Oistins and Bridgetown fish markets) and elsewhere, but has not been seen before or described in *Q. lugubris*. The Carib grackle is not the only innovative Quiscalus species: in North

America, the genus totals 19 innovations (Overington et al. 2009), making it the second most innovative Passerine genus after *Corvus* in that part of the world.

In field experiments, bullfinches and grackles were the fastest of five tested species (*Molothrus bonariensis*, *Zenaida aurita* and *Columbina passerina* were the others) to open a problem-solving apparatus (Webster and Lefebvre 2001). Bullfinches and grackles were also the least neophobic of the five species. Bullfinches further proved bolder than bananaquits *Coereba flaveola* in experiments where dishes of dissolved sugar were offered in the field (Webster and Lefebvre 2000). Barbados bullfinches take and pierce packets of refined sugar from restaurant tables (Reader et al. 2002; Ducatez et al. 2013; figure 5a). Investigations of this behaviour provide the first direct evidence of the independent emergence of the same behavioural innovation in different individuals in different places (Ducatez et al. 2013). Barbados bullfinches open the lids of sugar jars (figure 5b and SM movie 1), steal cream from jugs on terraces (figure 5c and SM movie 2) and reach for food in deep trash bins (figure 5d). Untrained individuals readily solve obstacle removal tasks in the wild (figures 6a and b). In captivity, both bullfinches and grackles perform well on problem-solving tasks like the two-step "tunnel task" (Audet et al. 2016a; figure 6c), where birds have to pull a stick out of a transparent tunnel to gain access to a plastic container and then flip a lid to obtain the reward, or the three-step "chest task" (figure 6d and SM movie 3), where the birds have to displace a wooden stick to unlock a metal latch, then push or pull to open the latch and finally push the base of the box to open it. Finally, both Barbados bullfinches and Carib grackles spontaneously solve the string-pulling test (Audet et al. 2016b; figure 6e), which is considered by some to involve an understanding of cause-effect relationships (Heinrich 1995; Emery and Clayton 2004; Werdenich and Huber 2006).

The ease with which Carib grackles and Barbados bullfinches can be tested in captivity has provided insights into differences in problem-solving between individuals and populations. At the population level, Barbados bullfinches from urbanised areas perform better in problem-solving tasks compared to rural individuals (Audet et al. 2016a); urban bullfinches are also bolder and have a stronger immune response than rural ones. At the individual level, Carib grackles that responded to movements of the

obstacle by redirecting their probes from the centre of the apparatus to its edges were more successful at solving the problem (Overington et al. 2011). Interestingly, individual differences in grackle obstacle removal performance are *negatively* correlated with discrimination learning performance: birds that are fast at obstacle removal are also fast at making discrimination choices, good or bad, making more errors in the process and thus reaching the learning criterion later (Ducatez et al. 2015). This surprising negative relationship between tasks can be reconciled as a coherent individual strategy that favours different aspects of a single speed-accuracy trade-off (Sih and Del Giudice 2012), where better problem solvers rapidly interact with a variety of stimuli that lead to obstacle removal (Overington et al. 2011), but also to higher error rates in situations where wrong choices are penalized.

One of the most intriguing opportunities offered by Emberizoid variation in innovativeness in Barbados is the sharp difference between *L. barbadensis* and its closest phylogenetic relative on the island (Burns et al. 2002; Burns et al. 2014; Barker et al. 2015), the black-faced grassquit *Tiaris bicolor*, a granivorous species that eats small seeds. Barbados bullfinches are extremely tame, neophilic and opportunistic, but grassquits, in contrast, do not approach novel patches of provisioned seed or anthropogenic sources of food (Kayello 2013). Both *L. barbadensis* and *T. bicolor* are territorial in Barbados and both feed on seeds in similar environments, but the sharp difference in their opportunism, if associated with differences in problem-solving (Sih and Del Giudice 2012), might yield important insights into the evolution of cognitive divergence between species otherwise matched for phylogeny, sociality and diet.

Conclusion

Our study provides clear evidence for high innovativeness at all levels of the nested phylogeny leading to Darwin's finches, with the family level providing the most robust results on both innovation frequency and rate corrected for research effort. This supports the suggestions independently derived from research on cognition (Tebbich et al. 2010), molecular phylogenetics (Burns et al. 2002; Mallarino et al. 2012; Burns et al. 2014) and taxonomic diversity (Ricklefs 2003) that the higher stems from which

Darwin's finches descend are also flexible. Observations and experiments in the field, as well as studies done in captivity, show that members of the *Emberizoidea* superfamily in Barbados are good model species for the experimental study of innovativeness and problem-solving. The high level of evolutionary radiation that accompanies behavioural plasticity in Galápagos finches does not characterize Lesser Antillean passerines in general (Ricklefs and Bermingham 2007), but rapid speciation does seem to have characterized the divergence of *L. barbadensis* from the *L. noctis* stem found on nearby islands (Buckley and Buckley 2004). Intriguingly, one of the key traits that differentiates *L. barbadensis* from *L. noctis* is shared with Barbados populations of *Q. lugubris fortirostris*: the two species have evolved monomorphic plumage in Barbados, while populations on other islands are sexually dimorphic. However, monomorphic plumage has evolved in different directions in the two species: *L. barbadensis* males have lost the black and red plumage that *L. noctis* shows on other islands and converged on the female's brown colouration, while *Q. lugubris fortirostris* females have lost the brown plumage they show on other islands and converged on the male's black.

The Emberizoids of Barbados, in particular *L. barbadensis* due to its close phylogenetic proximity with Darwin's finches, offer a unique opportunity to study the flexible stem. Barbados is more accessible and less ecologically fragile than the Galápagos. Many avian species are extremely tame there and adapt well to captive testing, and are thus ideal models to investigate variation in innovativeness, and more generally, cognition in wild birds. By combining experimental studies of wild birds kept in captivity for short periods of time and large scale comparative analyses quantifying innovative behaviours in the wild, we provide strong support for the flexible stem hypothesis. The high innovativeness and problem-solving abilities of *Emberizoidea* are likely to have been a major driver of the high diversification rate, adaptive radiation and colonisation abilities observed in this superfamily. High innovativeness is associated with high colonisation success across the entire class of birds (Sol et al. 2005) and the combination of the two might also have been a factor in the planetary radiation of the genus Homo (Lefebvre 2013).

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Figures

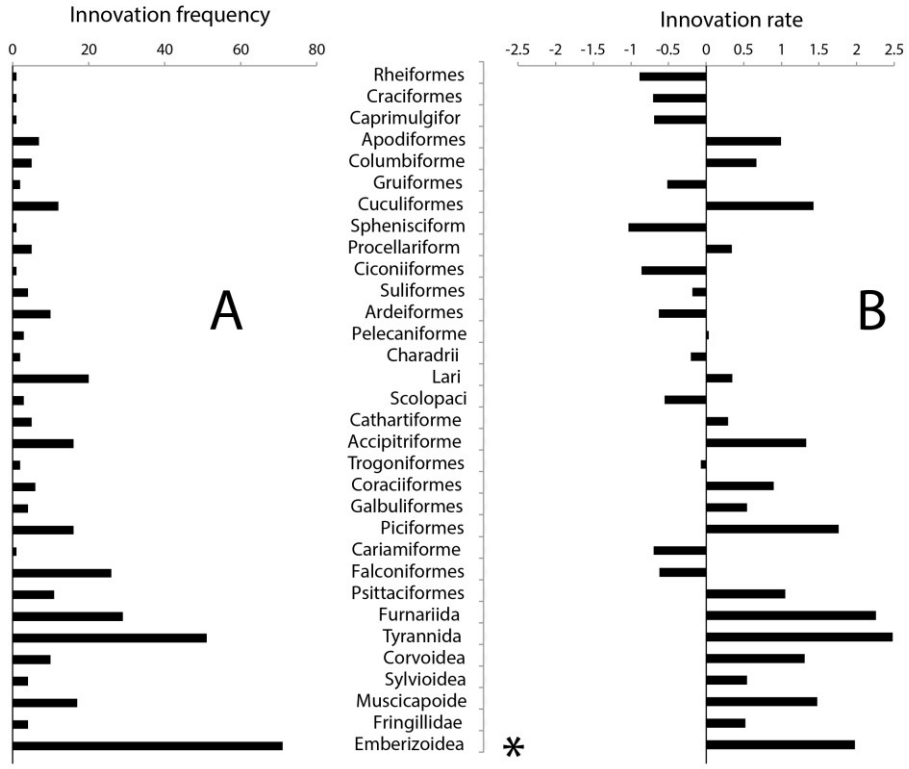

Figure 1. Frequency (A) and rate (B; residuals of frequency corrected by research effort and phylogeny) of feeding innovations in Neotropical orders and superfamilies.

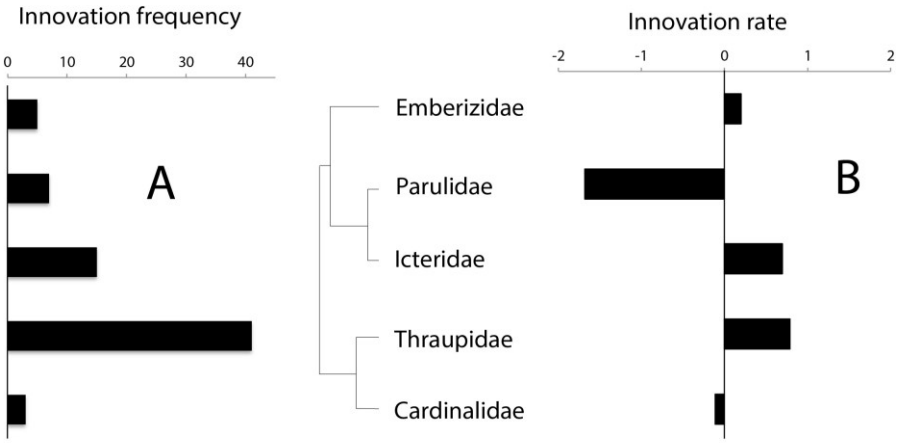

Figure 2. Frequency (A) and rate (B; residuals of frequency corrected by research effort and phylogeny) of feeding innovations in families of the superfamily Emberizoidea. The phylogenetic tree is adapted from [1].

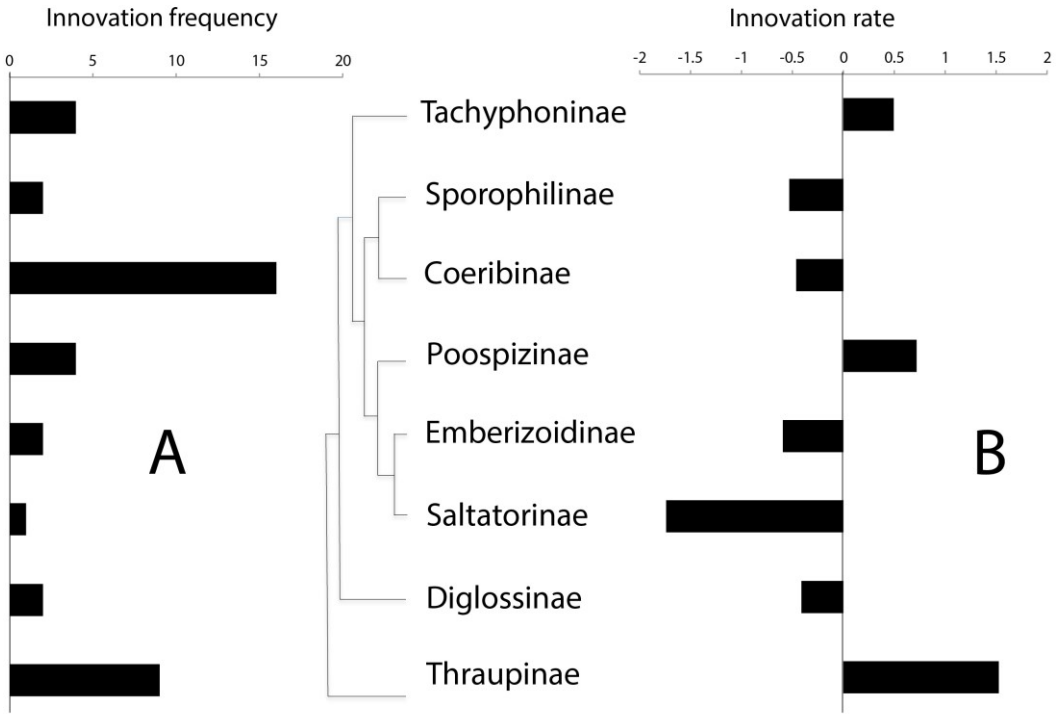

Figure 3. Frequency (A) and rate (B; residuals of frequency corrected by research effort and phylogeny) of feeding innovations in subfamilies of the family Thraupidae. The phylogenetic tree is adapted from [5].

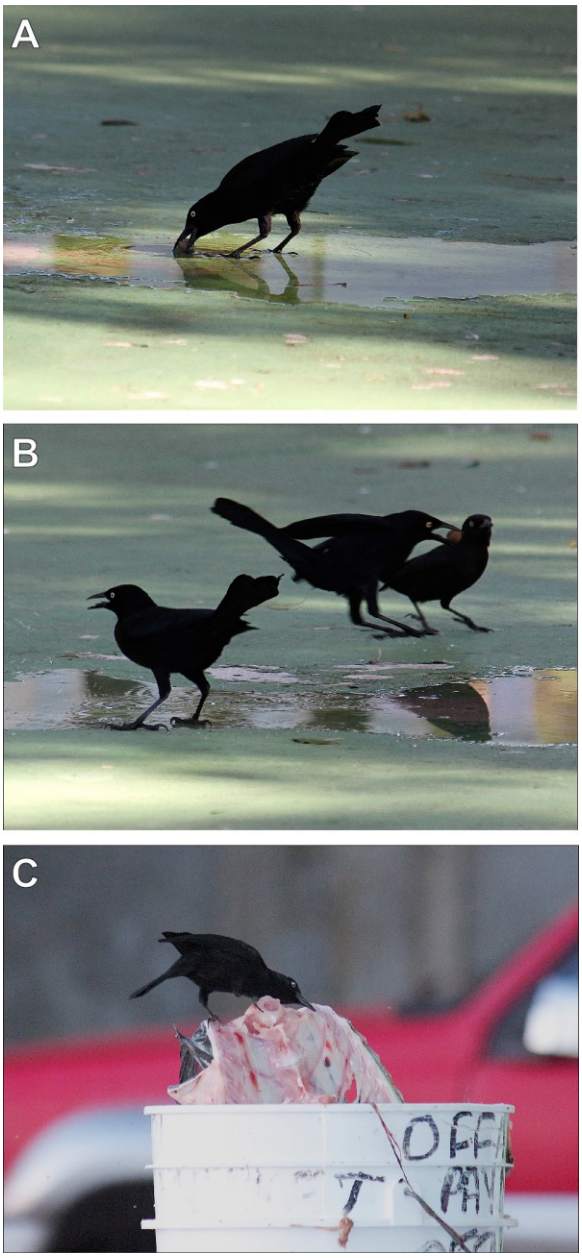

Figure 4. Feeding innovations in Carib grackles in Barbados. A: dunking dog pellets in water at the Bellairs Research Institute. B: stealing a pellet from a dunking bird. C: eating fish remains at the Payne's Bay fish market.

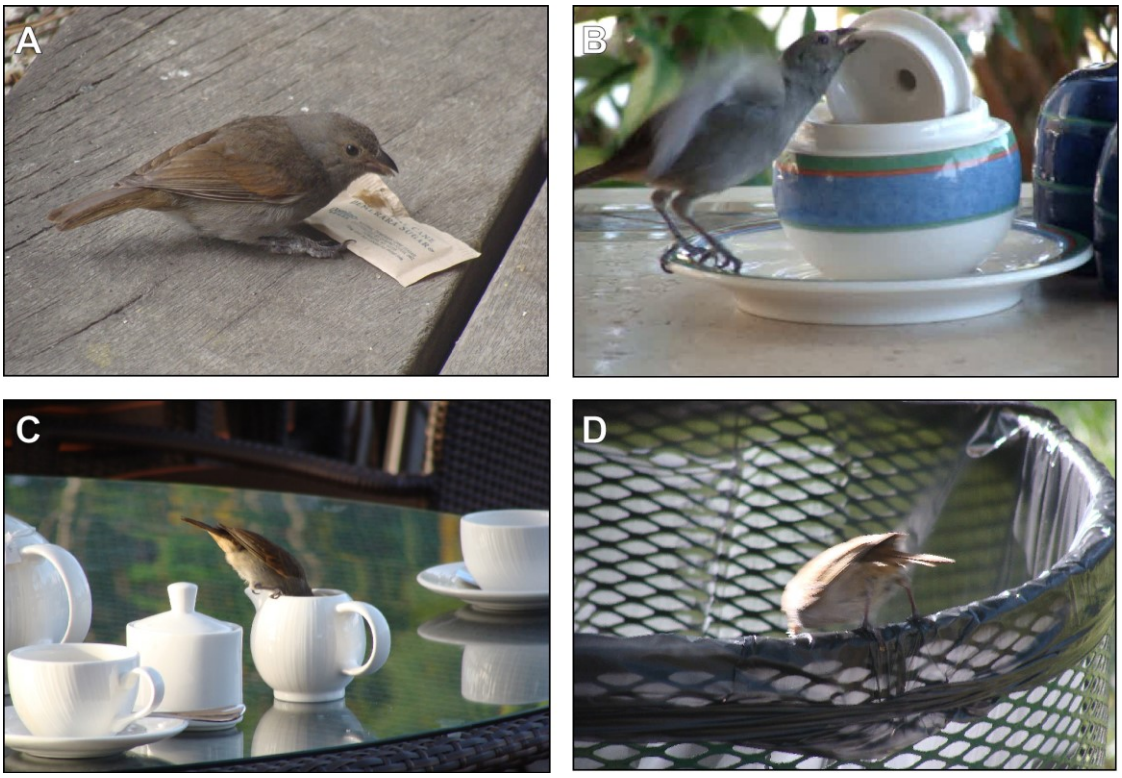

Figure 5. Feeding innovations and opportunistic feeding in Barbados bullfinches in the field. A: opening sugar packets at a restaurant. B: lifting the lid on a bowl of sugar (see also SM movie 1). C: drinking cream from a jug on a restaurant table (see also SM movie 2). D: foraging inside a garbage can.

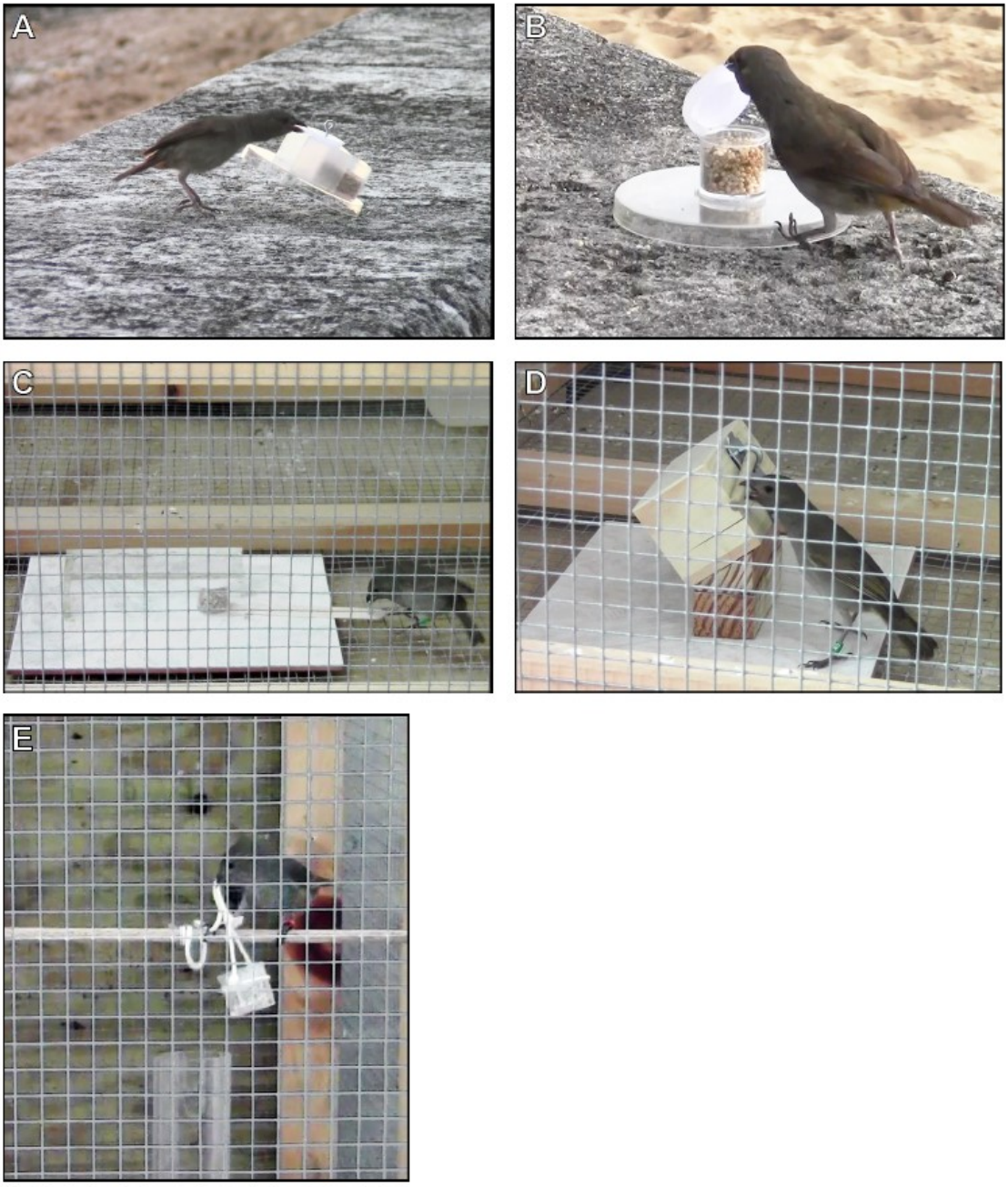

Figure 6. Problem-solving in Barbados bullfinches. A: opening a box containing seed in the field. B: lifting the lid on a cylinder containing seed in the field. C: in captivity, pulling a stick out of a tunnel to open a cylinder containing seed. D: opening the three-step chest task (see also SM movie 3). E: string pulling.

Supplementary material

Supplementary methods

We exhaustively searched the short notes sections of the following journals: *Anales del Instituto de Biología serie Zoología* (Mexico, 1991-2004); *Ararajuba*, followed by *Revista Brasileira de Ornitologia* (Brazil, 1990-2014; *Atualidades Ornitológicas* (Brazil, 1996-2014); *Aves Argentinas* (Argentina, 2012-2013); *Biota Neotropica* (Brazil, 2001-2015) *Biotemas* (Brazil, 1998- 2014); *Boletim CEO* (Brazil, 1986 - 2003); *Boletín Chileno de Ornitología* (Chile, 1994-2013); *Boletín de la Unión de Ornitólogos del Perú* (Peru, 2006-2014); *Boletín SAO* (Colombia, 1990-2015); *Brenesia* (Costa Rica, 2006-2011); *Bulletin of the British Ornithologist's Club* (worldwide, 1980-2014); *Caldasia* (Colombia, 1940-2014); *Churea* (Mexico, 2014-2015); *Cotinga* (Neotropics, 1994-2003, 2010-2013); *El Canto del Centzontle* (Mexico, 2010-2012); *El Hornero* (Argentina, 1941-2014); *El Pitirre*, followed by the *Journal of Caribbean Ornithology* (Caribbean, 1988-2002, 2011-2014); *Huitzil* (Mexico, 2000-2015); *Journal of Field Ornithology* (Neotropics, 1980-2014); *La Chiricoca* (Chile, 2006-2015); *La Tangara* (Nicaragua, 1999-2003); *Lundiana* (Brazil, 1980-2013); *Cormorant*, followed by *Marine Ornithology* (worldwide, 1978-2014); *Merganetta* (Colombia, 2007-2014); *Nattereria* (Brazil, 2000-2001); *Neotropical Birding* (Central and South America, Caribbean, 2006-2012); *Noticias de Galápagos* (Galápagos, 1963-2013); *Nuestras aves* (Argentina, 1962-2014); *Ornithologia* (Brasil, 2005-2014); *Ornitologia Colombiana* (Colombia, 2003-2014); *Ornitologia Neotropical* (Mexico, 1990-2006); *Pato Poc* (Guatemala, 2004-2006); *Revista Venezolana de Ornitologia* (Venezuela, 2011-2013); *Solitarius* (Belize, 2010-2013; *Spizaetus* (Neotropical raptors, 2005-2014); *Zeledonia* (Costa Rica, 1997-2014). In a few cases, articles in these journals referred to reports in ornithology journals that cover other areas of the world (*Wilson Journal of Ornithology, Condor, Emu*); we include these reports in Table 1.

The search was done before our predictions on the flexible stem were generated, so its results are unlikely to be biased *a priori* towards the hypothesis. We searched for key words such as 'unrecorded, 'opportunistic', 'not previously reported', 'new', 'not been documented', 'not found in the literature', 'depart markedly from their normal feeding habits', 'first record', 'never seen', 'only report is on other species', 'no reference', 'not previously published' etc. Each entry in SM Table 1 includes, after the semi-colon in the column describing the innovation, the keywords used by the original authors of the report. Translation from the Castilian or Portuguese of the original papers is by LL.

Research effort was taken from Ducatez and Lefebvre (2014) and supplemented, in the case of species whose names have changed, with searches in *The Zoological Record* (1978-2008) using previous names. In a few cases, research effort is likely inflated by the presence of species whose distribution goes far beyond the Neotropics (e.g. *Bubulcus ibis,* and *Ardea alba*). Residuals calculated against research effort are thus conservative in these cases.

Residuals of PGLS regressions of innovation frequency against research effort per clade were calculated using the procedure pgls from the R package “ape”, and time-calibrated ultrametric trees extracted from the phylogeny in Jetz et al. [6] at the superfamily and order level (Figure 1) or the phylogeny in Barker et al. [2] for analyses comparing the nine-primaried oscine families (Figure 2) and the Thraupid subfamilies (Figure 3). The phylogenies from Jetz et al. [6] and Barker et al. [2] do not provide a unique consensus tree, but sample trees from a pseudo-posterior distribution. We randomly extracted 50 different trees for each phylogenetic level and ran one PGLS model per tree. We then averaged the residuals and Pagel’s λ values over the 50 different phylogenies. Pagel’s λ were estimated from the PGLS regression using maximum likelihood. This parameter varies from 0 to 1, values close to 0 reflecting the absence of a phylogenetic signal, and values close to 1 reflecting a variance-covariance matrix following the Brownian model of evolution. All analyses were conducted using the packages “ape” and “caper in R 3.01.

Table S1. Neotropical innovations.

Higher level	Family	Species	Innovation	Reference
Rheiformes	Rheidae	Rhea americana	Fish in diet; unrecorded, opportunistic	Rev. Bras. Ornit. 2006 14, 285-287
Cracidae	Cracidae	Crax blumenbachii	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Caprimulgiformes	Caprimulgidae	Hydropsalis cayennensis	Using parking spot floodlight from which to pursue insects attracted to it; opportunistically, not been documented	J. Carib. Ornit. 2014 27, 40-41
Apodiformes	Apodidae	Streptoprocne zonaris	Use hilltop aggregation of horseflies and butterflies; locally abundant resources; not found in the literature.	Ornit. Neotrop. 2006 17, 619-622
Apodiformes	Apodidae	Streptoprocne zonaris	Feeding on winged termite swarm; unpredictable, never have been seen together, depart markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Apodiformes	Trochilidae	Eupetomena macroura	Feeding on urban termite swarm; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299
Apodiformes	Trochilidae	Eupherusa eximia	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Apodiformes	Trochilidae	Patagona gigas	Eating ashes and lime dust; no reports, first	Wilson J. 2008 120, 651–653
Apodiformes	Trochilidae	Chlorostilbon lucidus	Place feces on wall near nest, eat insects attracted; first report	Biotemas 2014 27, 201-203
Apodiformes	Trochilidae	Sephanoides sephanoides	Feeding on sap flow not caused by woodpecker; only report is on other species	Ornit. Neotrop. 2003 14, 531–533
Columbiformes	Columbidae	Patagioenas squamosa	Eat bread; not reported previously	El Pitirre 2002 15, 117-120
Columbiformes	Columbidae	Patagioenas squamosa	Feed on spilled maize at warehouse; never seen	Bull. Brit. Ornit. Club 2001 121, 247-249
Columbiformes	Columbidae	Columbina talpacoti	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Columbiformes	Columbidae	Geotrygon chiriquensis	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Columbiformes	Columbidae	Zenaida auriculata	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Ralli	Rallidae	Gallinula galeata	Feed on swarm of giant cicadas; opportunistic predators on seasonal abundant food source	Biota Neotrop. 2009 9, 259-262

Ralli	Rallidae	Gallinula galeata	Feeding on ticks and debris on body of posing capybara; new record	Biota Neotrop 2010 10, 195-203
Cuculiformes	Cuculidae	Crotophaga ani	Feed on swarm of giant cicadas; opportunistic predators on seasonal abundant food source	Biota Neotrop. 2009 9, 259-262
Cuculiformes	Cuculidae	Crotophaga ani	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Cuculiformes	Cuculidae	Crotophaga ani	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Cuculiformes	Cuculidae	Crotophaga ani	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Cuculiformes	Cuculidae	Crotophaga sulcirostris	Preys on pigeon egg; should be added to growing list, no reference could be found, only on other Ani species	Ornit. Neotrop. 2000 11, 231–232
Cuculiformes	Cuculidae	Guira guira	Feed on swarm of giant cicadas; opportunistic predators on seasonal abundant food source	Biota Neotrop. 2009 9, 259-262
Cuculiformes	Cuculidae	Guira guira	Predation on poisonous toad Rhinella granulosa; first record, refined handling technique not usual	Rev. Bras. Ornit. 2009 17, 84-85
Cuculiformes	Cuculidae	Guira guira	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Cuculiformes	Cuculidae	Guira guira	Prey on iguana; large, first record	Rev. Bras. Ornit. 2014 22, 305-306
Cuculiformes	Cuculidae	Guira guira	Feeding on wasps outside nests; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Cuculiformes	Cuculidae	Piaya cayana	Feed on swarm of giant cicadas; opportunistic predators on seasonal abundant food source	Biota Neotrop. 2009 9, 259-262
Cuculiformes	Cuculidae	Piaya cayana	Feeding on wasps outside nests; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Sphenisciformes	Spheniscidae	Pygoscelis papua	Closely-concerted small group feeding on krill; not recorded in recent times, none had ever observed this type of behavior before	Mar. Ornit. 2008 36, 193–194
Procellariformes	Diomedeidae	Thalassarche melanophrys	Eats storm petrel; first time	Mar. Ornit. 2008 36, 77–78
Procellariformes	Diomedeidae	Thalassarche melanophrys	Eats tern; first reported	Mar. Ornit. 2006 34, 167–168
Procellariformes	Diomedeidae	Phoebastria irrorata	Scavenge food regurgitated/dropped by boobies harassed by frigatebirds at feeding frenzy; unpublished, take advantage	Not. Galap. 1998 59, 20-23
Procellariformes	Procellariidae	Macronectes giganteus	Same individual preys on adult cormorants; not previously published	Mar. Ornit. 1995 23, 166-167
Procellariformes	Hydrobatidae	Oceanodroma melania	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Ciconiiformes	Ciconiidae	Mycteria americana	Predation of swamp eel (specialiity of Jabiru)	Atual. Ornit.2013 175, 25
Suliformes	Phalacrocoracidae	Phalacrocorax auritus	Use dolphin group herding of fish to dive in swarm; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Suliformes	Phalacrocoracidae	Phalacrocorax penicillatus	Use dolphin group herding of fish to dive in swarm; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262

Suliformes	Sulidae	Sula leucogaster	Use dolphin group herding of fish to dive in swarm; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Suliformes	Sulidae	Sula nebouxii	Use dolphin group herding of fish to dive in swarm; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Ardeiformes	Ardeidae	Bubulcus ibis	Feeding on crickets under a street light; opportunistically, not been documented	J. Carib. Ornit.2014 27, 40-41
Ardeiformes	Ardeidae	Bubulcus ibis	Predation on Cocos finch; first report	Zeledonia 2014 18, 94-96
Ardeiformes	Ardeidae	Bubulcus ibis	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Ardeiformes	Ardeidae	Butorides striata	Feed on swarm of giant cicadas; opportunistic predators on seasonal abundant food source	Biota Neotrop. 2009 9 259-262
Ardeiformes	Ardeidae	Butorides virescens	Prey on village weaver chicks at nest; not been reported	El Pitirre 14, 130-132, 2001
Ardeiformes	Ardeidae	Egretta thula	Picking invertebrates and catching horseflie on capybara in mud puddle; new record	Biota Neotrop. 2010 10, 195-203
Ardeiformes	Ardeidae	Tigrisoma lineatum	Bait fish with bread; tool	Ararajuba 1991 2, 89-90
Ardeiformes	Ardeidae	Ardea alba	Use otter disturbance in river to prey on fish; novel, unrecorded, learned, commensal	Bull. Brit. Orni. Club 1996 116, 199-200
Ardeiformes	Ardeidae	Ardea herodias	Preying on young turtle in Galápagos; not mentioned	Bull. Brit. Orni. Club 1995 115, 68
Ardeiformes	Ardeidae	Nyctanassa violacea,	Preying on turtle eggs; literature does not mention	Zeledonia 2006 10(2), 53-55
Pelecaniformes	Pelecanidae	Pelecanus occidentalis	Use dolphin group herding of fish to dive in swarm; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Pelecaniformes	Pelecanidae	Pelecanus occidentalis	Dipping feeding technique; unusual, not commonly reported, developed skill, possibly emulating ducks or waders	Cotinga 1997 9, 83
Pelecaniformes	Threskiornithidae	Theristicus melanopis	Feed on garbage and carrion; other foods mentioned in litterature	Nuestras Aves 1998 38, 12
Charadrii	Charadriidae	Charadrius semipalmatus	Use sediments overturned by shellfishers; profiting opportunistically, short-term learning	Emu 2014 114, 50-60
Charadrii	Charadriidae	Vanellus chilensis	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Lari	Laridae	Thalasseus elegans	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Lari	Laridae	Thalasseus maximus	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Lari	Laridae	Sterna hirundinacea	Prey on katydid; first record, opportunistically	Mar. Ornit. 2103 41, 199–200
Lari	Laridae	Larus atlanticus	Feed on grain; unknown, normally crab specialist	El Hornero 2007 22, 51-54
Lari	Laridae	Larus atlanticus	Exploit discards from high-seas fisheries; first record, normally crab specialist, novel shift in foraging, take advantage of abundant resource	El Hornero 2011 24, 105-109

Lari	Laridae	Larus californianus	Use dolphin group herding of fish to dive in swarm; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Lari	Laridae	Larus dominicanus	Kleptoparasitism of Magellanic Flightless Steamer-ducks; rare opportunistic occurrence	Boletín SAO 2007 17, 141-144
Lari	Laridae	Larus dominicanus	Kleptoparasitism on Royal and Cayenne Tern; first time	J. Field. Ornit. 70, 337-342, 1999
Lari	Laridae	Larus dominicanus	Kleptoparasitism on Common Tern and South American Tern; new record	Mar. Ornit. 2009 37, 291–292
Lari	Laridae	Larus dominicanus	Attack duck in lagoon; lack of sufficent resources may have forced to develop hunting behaviors	Nuestras Aves 2013 58, 36-37
Lari	Laridae	Larus dominicanus	Feeding on crustaceans on snout of right whale; new record	Biota Neotrop. 2010 10, 195-203
Lari	Laridae	Larus heermanni	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Lari	Laridae	Larus livens	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Lari	Laridae	Larus maculipennis	Nocturnal feeding by artificial light; opportunistic other gulls mentioned	El Hornero 2010 25, 55-60
Lari	Laridae	Larus maculipennis	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Lari	Laridae	Larus maculipennis	Feed on larvae and beetles behind tractor; first identification, opportunism generated by human activity	Bol. Chil. Ornit. 2008 14, 112-115
Lari	Stercorariidae	Stercorarius maccormicki	Attack from above in flight of snow petrel, drives it to ground; first published instance	Mar. Ornit. 2004 32, 115-116
Lari	Stercorariidae	Stercorarius antarcticus	Drinks blood from wound on back of elephant seal; new record	Biota Neotrop. 2010 10, 195-203
Lari	Stercorariidae	Stercorarius parasiticus	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Lari	Stercorariidae	Stercorarius pomarinus	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Scolopaci	Scolopacidae	Phalaropus lobatus	Use dolphin group herding to feed on chunks of fish; opportunistic, commensal	Ann. Inst. Biol. UNAM Zool. 1991 62, 253-262
Scolopaci	Scolopacidae	Calidris pusilla	Use sediments overturned by shellfishers; profiting opportunistically, short-term learning	Emu 2014 114, 50-60
Scolopaci	Scolopacidae	Arenaria interpres	Feed on insects in flowers, pollinate it; we were surprised	Not. Galap. 1993 52, 5-10
Cathartiformes	Catharthidae	Cathartes aura	Feed on Elaeis guineensis palm seed; new hábito, recent acquisition, capacity for adaptation	El Hornero 1965 10, 276-277
Cathartiformes	Catharthidae	Cathartes melambrotus	Feeding on remains of three-toed sloth; no published information	El Hornero 1992 13, 235
Cathartiformes	Catharthidae	Coragyps atratus	Learned to feed from plastic bags on beaches by generalizing from to carrion to garbage	Rev. Bras. Ornit. 2007 15, 617-620
Cathartiformes	Catharthidae	Coragyps atratus	Pick organic debris from the hair of a domestic dog; no () recorded, novelty	Rev. Bras. Ornit. 2010 18, 45-48; Biota Neotrop. 2010 10, 203

Cathartiformes	Catharthidae	Coragyps atratus	Nocturnal feeding by a group in a limestone quarry; no published reports	J Raptor Research 2011 45, 279-280
Accipitriformes	Accipitridae	Accipiter superciliosus	Predation on woodpecker; not previously reported, normally preys on hummingbirds	Cotinga 2011 33, 89–90
Accipitriformes	Accipitridae	Busarellus nigricollis	First published record of predation on Pantanal alligator	Rev. Bras. Ornit. 2012 20, 73-74
Accipitriformes	Accipitridae	Buteo polyosoma	Group hunting; intriguing, typically solitary hunter, first evidence, unusual	Ornit. Neotrop. 2005 16, 271–275
Accipitriformes	Accipitridae	Buteogallus urubitinga	Preys on tiger heron; one of the first specific reports, normal prey are frogs and lizards	Ornit. Neotrop. 1991 2, 37
Accipitriformes	Accipitridae	Buteogallus anthracinus	Preys on snake eel; first report	Brenesia 2010 73-74, 157-159
Accipitriformes	Accipitridae	Elanus leucurus	Eating leaves of Alnus acuminata; first report	Boletín SAO 2011 20, 46-51
Accipitriformes	Accipitridae	Harpia harpyja	First observed predation on an infant Capuchin Monkey	Rev. Bras. Ornit. 2010 18, 352-354
Accipitriformes	Accipitridae	Parabuteo unicinctus	Prey on bats at Mexico city supermarket; flexible, unusual, novel food, first formal record, urban adaptation	Rev. Bras. Ornit. 2014, 22, 297-299
Accipitriformes	Accipitridae	Parabuteo unicinctus	Prey on amphibians; not listed as part of the diet	Nuestras Aves 2012 57, 21-23
Accipitriformes	Accipitridae	Parabuteo unicinctus	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Accipitriformes	Accipitridae	Pseudastur albicollis	Prey on birds; literature does not report, considered to specialize on reptilian prey	Ornit. Neotrop. 2003 14, 541-543
Accipitriformes	Accipitridae	Rostrhamus sociabilis	Preys on turtle and crabs; normally snail specialist	Nuestras Aves 2009 54, 47-48
Accipitriformes	Accipitridae	Rupornis magnirostris	Takes passerine from mist net; oportunistic	Atual. Ornit.2009 151, 22
Accipitriformes	Accipitridae	Rupornis magnirostris	Predation on Bogota rail; first record, mainly takes small prey	Cotinga 2012 34, 94–95
Accipitriformes	Accipitridae	Spizaetus ornatus	Predation on Long–tailed Silky Flycatcher; first prey item reported	Rev. Bras. Ornit. 2012, 20, 451-452
Accipitriformes	Accipitridae	Spizaetus ornatus	Scavenge cattle femur; first observations, formerly assumed only to prey on live food	Rev. Bras. Ornit. 2014, 22, 27-31
Trogoniformes	Trogonidae	Trogon surrucura	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Trogoniformes	Trogonidae	Pharomachrus auriceps	Eat lizard; normally frugivorous, unusual	Cotinga 1998 9, 41
Coraciiformes	Momotidae	Eumomota superciliosa	Feed on insects attracted to artificial light at night; no species in this family previously reported	Wilson J. 2002 114, 525-526
Coraciiformes	Momotidae	Momotus momota	Preys on Akodon affinis; no report of its taking a mammal	.Ornit. Colomb. 2003 1, 63-65.
Coraciiformes	Momotidae	Momotus momota	Nocturnal foraging on moths; first observation, not previously recorded	Wilson J 2008 120, 653-654

Coraciiformes	Momotidae	Momotus momota	Predation on Sporophila and Alfaro's rice rat; new vertebrate prey, no other published reports	Zeledonia 2010 14(2), 68-72
Coraciiformes	Momotidae	Momotus momota	Feeding on wasps outside nests; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Coraciiformes	Momotidae	Momotus momota	Prey on masrsupial; first time, largest known prey	Ornit. Colomb. 2012 12, 51-53
Galbuliformes	Bucconidae	Nystalus chacuru	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Galbuliformes	Bucconidae	Nystalus maculatus	Predation on Chironius snake; first report	Ararajuba 1996 4, 113
Galbuliformes	Galbuliade	Galbula ruficauda	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Galbuliformes	Galbulidae	Galbula ruficauda	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Piciformes	Picidae	Celeus flavescens	Nectar feeding; normally insects and fruit	Biota Neotrop. 2006 6, 1-4
Piciformes	Picidae	Colaptes campestris	Feeding on wasps outside nests; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Piciformes	Picidae	Colaptes campestris	Feed on mandarin oranges; no reference	Nuestras Aves 2001 42, 22
Piciformes	Picidae	Colaptes melanochloros	Feed on mandarin oranges; no reference	Nuestras Aves 2001 42, 22
Piciformes	Picidae	Colaptes melanochloros	Feeding on urban termite swarm; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299
Piciformes	Picidae	Dryocopus lineatus	Eat fruit of Myrsine coriacea; no records in literature of frugivory	Biotemas 2013 26, 261-263 2013
Piciformes	Picidae	Melanerpes candidus	Feed on meat and fat from sheep	Atual. Ornit. 2013 176, 10-11
Piciformes	Picidae	Melanerpes hoffmannii	Storing insects in tree trunk; first time I saw, surprising	Zeledonia 2013 17(2), 72-74
Piciformes	Picidae	Melanerpes rubricapillus	Insert fruit in fence post, use as vice tool; singular, not previously reported	Rev. Venez. Ornit.2013 3, 46–48
Piciformes	Picidae	Piculus aurulentus	Fruit consumption; first record	Lundiana 2008 9, 159-160
Piciformes	Ramphastidae	Andigena laminirostris	Eating Caecilia amphibian; opportunistic, not in regular diet	Boletín SAO 2011 20, 43-45
Piciformes	Ramphastidae	Pteroglossus bailloni	Preys on woodpecker nestlings, gets stuck in nest; not registered for this species, other toucans prey on nestlings	Nuestras Aves 2004 47, 16-17
Piciformes	Ramphastidae	Ramphastos vitellinus	Predation by battering of black rat; never been reported	Rev. Bras. Ornit. 2012 20, 156-157
Piciformes	Ramphastidae	Ramphastos dicolorus	Predation on rufous bellied thrush; basically frigivorous, predation mentioned in other Ramphastos species	Atual. Ornit.2010 158, 55-56
Piciformes	Ramphastidae	Ramphastos ambiguus	Preys on rodent; first report, very unusual, considered frugivorous	Zeledonia 2014 18(2), 67-69

Piciformes	Ramphastidae	Ramphastos ambiguus	Preys on bats; not registered up to now	Zeledonia 2007 11(2), 24
Cariamiformes	Cariamidae	Chunga burmeisteri	Smash food items on anvils; tool, novel, interesting	Rev. Bras. Ornit. 2014 22, 234-237
Falconiformes	Falconidae	Caracara plancus	Feeding on bacuri palm Attalea phalerata; other foods listed as known	Ararajuba 2004 12, 133-135
Falconiformes	Falconidae	Caracara plancus	Kleptoparasitism on Harris Hawks and on conspecifics at henhouse, slaughterhouse and garbage; additonal information, other species known	J. Field. Ornit. 1992 63, 177-180
Falconiformes	Falconidae	Caracara plancus	Cleaning capybaras; new records	Biota Neotrop. 2010 10, 195-203
Falconiformes	Falconidae	Caracara plancus	Foraging on swarming leafcutter ants; previously unrecorded	Rev. Bras. Ornit. 2007 15, 592-597
Falconiformes	Falconidae	Caracara plancus	Waiting for organic human refuse; previously unrecorded	Rev. Bras. Ornit. 2007 15, 592-597
Falconiformes	Falconidae	Caracara plancus	Pair and juvenile prey on adult cattle egret, team work	Bolletin SAO 7 (12-13), 73, 1996
Falconiformes	Falconidae	Caracara plancus	Predation on amphibian	Atual. Ornit.2012 169, 12-13
Falconiformes	Falconidae	Daptrius ater	Foraging on live small fish; unreported, adds another feeding mode	Biota Neotrop. 2009 9, 400-401
Falconiformes	Falconidae	Daptrius ater	Cleaning capybaras; new records	Biota Neotrop. 2010 10, 195-203
Falconiformes	Falconidae	Falco femoralis	Predation on Cattle Egret and campo flicker; normally much smaller prey	Rev. Bras. Ornit. 2006 14, 453-454
Falconiformes	Falconidae	Falco femoralis	Steal crayfish from Little Blue Heron; first instance, no records	J. Field. Ornit. 60, 380-381, 1989
Falconiformes	Falconidae	Falco femoralis	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Falconiformes	Falconidae	Falco peregrinus	Dive for fish; first record of intentional full diving	Rev. Bras. Ornit. 2013 21, 217-220
Falconiformes	Falconidae	Falco peregrinus	Attack on 3 large gull species; no previous reports of hunting on birds of that size	Boletin UNOP 2010 5, 12-13
Falconiformes	Falconidae	Falco sparverius	Predation on bats as they left their roost in late evening in Marie Galante; first report, adaptation	J Raptor Research 2014 48, 78-81
Falconiformes	Falconidae	Falco sparverius	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Falconiformes	Falconidae	Herpetotheres cachinnans	Perched on back of cow, possibly in search of ectoparasites	Zeledonia 1999 3(2), 1-8
Falconiformes	Falconidae	Ictinia plumbea	Adults eat fruit and dead squirrel cuckoo, feed to juvenile; literature does not record consumption of dead prey	Biotemas 2011 24, 77-82
Falconiformes	Falconidae	Milvago chimachima	Inspecting a tapir in search of ticks; new record; also three toed sloth, opportunistic, undescribed	Biota Neotrop. 2010 10, 195-203 J. Rapt. Res. 1999 33, 2
Falconiformes	Falconidae	Milvago chimachima	Predation on turtle	Atual. Ornit. 2008 142, 22

Falconiformes	Falconidae	Milvago chimachima	Demonstrates hunting techniques with stones and vegetation in front of young; transmission of information, learning	Zeledonia 2014 18(2), 62-66
Falconiformes	Falconidae	Milvago chimachima	Biting back of iguanas, looking for ectoparasites	Zeledonia 1999 3(2), 1-8
Falconiformes	Falconidae	Milvago chimango	Fishing using ‘glide-hover’ technique; unreported	Biota Neotrop. 2009 9, 403-405
Falconiformes	Falconidae	Milvago chimango	Feeding on ticks of a posing capybara; new record	Biota Neotrop. 2010 10, 195-203
Falconiformes	Falconidae	Milvago chimango	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Falconiformes	Falconidae	Milvago chimango	Scavenge food from red-backed hawks; one of few cases, combined with their opportunistic and innovative behaviour	Emu 2009 109, 260–264
Falconiformes	Falconidae	Phalcoboenus australis	Picks mucus from nostril of elephant seal; new record	Biota Neotrop. 2010 10, 195-203
Psittaciformes	Psittacidae	Anodorhynchus leari	Attack corn plantations as a result of low production of licuri palm trees	Ornithologia 2007 2, 41-46
Psittaciformes	Psittacidae	Anodorhynchus leari	Eating snail and mandacaru fruit (in period of low licuri productivity); unpublished, recorded for first time	Atual. Ornit. 2014 178, 50-54
Psittaciformes	Psittacidae	Anodorhynchus hyacinthinus	Use cattle (and maybe extinct megafauna before) digestion to obtain palm nut seeds without fruit	Ararajuba 1997 5, 176-182
Psittaciformes	Psittacidae	Ara ararauna	Feed on plants in firebreaks; adds six species to known diet, not noted by previous studies	Biotemas 2009 22, 105-115
Psittaciformes	Psittacidae	Cyanoliseus patagonus	Feed on roble seeds; opportunist, document for the first time, novelty, has not been described	Bol. Chil. Ornit. 2010 16, 17-20
Psittaciformes	Psittacidae	Eupsittula aurea	Feeding on arboreal and alate termites; unpredictable, ephemeral, opportunistic	Wilson J 1989 101, 656-657; Rev. Bras. Ornit. 2007 15, 458
Psittaciformes	Psittacidae	Myiopsitta monachus	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Psittaciformes	Psittacidae	Pyrrhura devillei	Report for the first time the chewing of bromeliad leaves for water	Rev. Bras. Ornit. 2009 17, 210-212
Psittaciformes	Psittacidae	Pyrrhura frontalis	High intake of leaves in the diet; not previously recorded for any species of Neotropical psittacids.	Ornit. Neotrop. 2001 12, 215–223
Psittaciformes	Psittacidae	Brotogeris tirica	Feed on palm Syagrus romanzoffiana; first substantiated record, unrecorded	Biota Neotrop. 2008 8, 231-234
Psittaciformes	Psittacidae	Pyrilia haematotis	Feed on mistletoe; opportunistic, only published dietary notes are other foods	Ornit. Neotrop. 1994 5, 119-120
Furnariida	Formicariidae	Formicarius rufipectus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Furnariida	Furnariidae	Anumbius annumbi	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Furnariida	Furnariidae	Sittasomus griseicapillus	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Furnariida	Furnariidae	Certhiaxis cinnamomeus	Preys on fish; first data	Nuestras Aves 2010 55, 17-19

Furnariida	Furnariidae	Cinclodes antarcticus	Feeding on blood from wounded nose and body of elephant seals; new record	Biota Neotrop. 2010 10, 195-203
Furnariida	Furnariidae	Cinclodes olrogi	Preys on tadpole, feeds to young; first data	Nuestras Aves 2010 55, 17-19
Furnariida	Furnariidae	Cinclodes atacamensis	Preys on tadpole; first data	Nuestras Aves 2010 55, 17-19
Furnariida	Furnariidae	Drymornis bridgesii	Preys on small lizard and passerine egg; not mentioned	Nuestras Aves 2003 46, 45-47
Furnariida	Furnariidae	Furnarius rufus	Feeding on urban termite and swarms; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299; Nuestras Aves 201 63-64
Furnariida	Furnariidae	Furnarius rufus	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Furnariida	Furnariidae	Furnarius rufus	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Furnariida	Furnariidae	Furnarius rufus	Preys on fish and tadpoles; first data	Nuestras Aves 2010 55, 17-19
Furnariida	Furnariidae	Furnarius rufus	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Furnariida	Furnariidae	Margarornis rubiginosus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Furnariida	Furnariidae	Philydor rufum	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Furnariida	Furnariidae	Pseudoseisura lophotes	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Furnariida	Furnariidae	Synallaxis spixi	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Furnariida	Furnariidae	Synallaxis spixi	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Furnariida	Furnariidae	Thripadectes rufobrunneus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Furnariida	Furnariidae	Xenops rutilans	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Furnariida	Furnariidae	Synallaxis albescens	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Furnariida	Furnariidae	Xiphocolaptes albicollis	Prey on young passerine; not mentioned	Nuestras Aves 2003 46, 45-47
Furnariida	Furnariidae	Xiphocolaptes major	Prey on bat, bash against trunk; opportunist, eats arthropods	Nuestras Aves 2003 46, 45-47
Furnariida	Furnariidae	Lepidocolaptes squamatus	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Furnariida	Furnariidae	Xiphorhynchus fuscus	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Furnariida	Thamnophilidae	Frederickena viridis	Prey on paper wasp nest by hitting it; first record	Rev. Bras. Ornit. 2014 22, 300-302

Furnariida	Thamnophilidae	Myrmeciza loricata	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Furnariida	Thamnophilidae	Drymophila ochropyga	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Furnariida	Thamnophilidae	Thamnophilus caerulescens	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Tyrannida	Cotingidae	Schiffornis virescens	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Tyrannida	Cotingidae	Ampelioides tschudii	Eat arboreal snail; amazing, wow (comments on Flicker photos)	Cotinga 2002 18, 100
Tyrannida	Cotingidae	Phytotoma raimondii	Feed on exotic Tamarix plant; first report	Boletin UNOP 2013 8, 16-24
Tyrannida	Pipridae	Chiroxiphia linearis	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Tyrannida	Pipridae	Piprites pileata	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Tyrannida	Pipridae	Ilicura militaris	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Tyrannida	Pipridae	Neopelma pallescens	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Tyrannida	Tyrannidae	Empidonax flavescens	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Tyrannida	Tyrannidae	Agriornis lividus	Preys on house sparrow	Bol. Chil. Ornit. 1995 2, 28
Tyrannida	Tyrannidae	Camptostoma obsoletum	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Empidonomus varius	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Griseotyrannus aurantioatrocristatus	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Tyrannida	Tyrannidae	Legatus leucophaius	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Myiodynastes maculatus	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Myiozetetes similis	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Megarynchus pitangua	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Lathrotriccus euleri	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Myiophobus fasciatus	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Elaenia flavogaster	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71

Tyrannida	Tyrannidae	Elaenia albiceps	Eat nectar from exotic flower; great plasticity, adapted	Bol. Chil. Ornit. 2002 9, 25-26
Tyrannida	Tyrannidae	Fluvicola nengeta	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Tyrannida	Tyrannidae	Gubernetes yetapa	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Tyrannida	Tyrannidae	Hirundinea ferruginea	Use hilltop aggregation of horseflies and butterflieslocally abundant resources; not found in the literature.	Ornit. Neotrop. 2006 17, 619-622
Tyrannida	Tyrannidae	Machetornis rixosa	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Tyrannida	Tyrannidae	Mionectes olivaceus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Tyrannida	Tyrannidae	Mionectes rufiventris	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Tyrannida	Tyrannidae	Muscipipra vetula	Use hilltop aggregation of horseflies and butterflieslocally abundant resources; not found in the literature.	Ornit. Neotrop. 2006 17, 619-622
Tyrannida	Tyrannidae	Muscipipra vetula	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Tyrannida	Tyrannidae	Muscipipra vetula	Taking insects from mammal feces on road; curious	Atual. Ornit.2012 166, 6-8
Tyrannida	Tyrannidae	Myiarchus swainsoni	Use hilltop aggregation of horseflies and butterflies; locally abundant resources, not found in the literature.	Ornit. Neotrop. 2006 17, 619-622
Tyrannida	Tyrannidae	Myiarchus swainsoni	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Myiarchus tyrannulus	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Phylloscartes ventralis	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Tyrannida	Tyrannidae	Pitangus sulphuratus	Steals from conspecific; not reported in family	El Hornero 1992 13, 234-235
Tyrannida	Tyrannidae	Pitangus sulphuratus	Take bats from openings in building; never been reported	J Field Orni2010 81, 17–20
Tyrannida	Tyrannidae	Pitangus sulphuratus	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Tyrannida	Tyrannidae	Pitangus sulphuratus	Predation on Amazon pufferfish; surprises, enigmatic, known to be toxic, do not mention	Rev. Bras. Ornit. 2009 17, 77-78
Tyrannida	Tyrannidae	Pitangus sulphuratus	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Tyrannida	Tyrannidae	Pitangus sulphuratus	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Tyrannida	Tyrannidae	Tyrannopsis sulphurea	Attempt to steal food from boat-billed flycatcher; no reports of interspecific kleptoparasitism	Ornit. Neotrop. 1998 9, 93–94
Tyrannida	Tyrannidae	Tyrannus albogularis	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92

Tyrannida	Tyrannidae	Tyrannus dominicensis	Take bread from ground; not reported	Bull. Brit. Orni. Club 2001 121, 247-249
Tyrannida	Tyrannidae	Tyrannus dominicensis	Hawk dog pellet from ground, batter on tree; not reported	El Pitirre 15, 117-120, 2002
Tyrannida	Tyrannidae	Tyrannus melancholicus	Intraspecific theft of large insect; only occasion	El Pitirre 13, 7, 2000
Tyrannida	Tyrannidae	Tyrannus melancholicus	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92; Nuestras Aves 2013 58, 6
Tyrannida	Tyrannidae	Tyrannus melancholicus	Feeding on winged termite and ant swarms; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Tyrannus savana	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Tyrannida	Tyrannidae	Tyrannus savana	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Tyrannida	Tyrannidae	Alectrurus risora	Use swarms of army ants (also armadillos) to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Tyrannida	Tyrannidae	Xolmis cinereus	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Tyrannida	Tyrannidae	Myiozetetes similis	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Corvoidea	Corvidae	Cyanolyca cucullata	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Corvoidea	Corvidae	Cyanocorax cristatellus	Prey on lizard; first observation, no reptiles reported, newly-discovered food	Biotemas 2009 22, 243-245
Corvoidea	Corvidae	Cyanocorax cyanomelas	Taking small pieces of meat hung in the open to dry, ingesting remains of meat adhering to cattle skin drying in the sun	Lundiana 2005 6, 57-66
Corvoidea	Corvidae	Calocitta formosa	Biting the back of a deer, possibly looking for ectoparasites	Zeledonia 1999 3(2), 1-8
Corvoidea	Corvidae	Psilorhinus morio	Preying on dove; no one has observed	Zeledonia 2008 12(1), 38-39
Corvoidea	Vireonidae	Cyclarhis gujanensis	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Corvoidea	Vireonidae	Cyclarhis gujanensis	Preys on bat, warbler, bashes grass snake; known diet insects, larvae, fuit	Nuestras Aves 2010 54, 43-45
Corvoidea	Vireonidae	Hylophilus amaurocephalus	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Corvoidea	Vireonidae	Vireo olivaceus	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Corvoidea	Vireonidae	Hylophilus poicilotis	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Sylvioidea	Hirundinidae	Notiochelidon cyanoleuca	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Sylvioidea	Hirundinidae	Notiochelidon cyanoleuca	Use hilltop aggregation of horseflies and butterflies; locally abundant resources, not found in the literature.	Ornit. Neotrop. 2006 17, 619-622

Sylvioidea	Hirundinidae	Progne chalybea	Taking insects from mammal feces on road; curious	Atual. Ornit.2012 166, 6-8
Sylvioidea	Hirundinidae	Progne tapera	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Muscicapoidea	Troglodytidae	Troglodytes musculus	Feeding on urban termite swarm; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299
Muscicapoidea	Troglodytidae	Troglodytes ochraceous	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Muscicapoidea	Troglodytidae	Cyphorhinus phaeocephalus	Prey on small frog, bash it against branch; new observation	Zeledonia 2001 5, 8-9
Muscicapoidea	Mimidae	Mimus saturninus	Predation on watersnake; no species of Mimus is reported as preying on snakes	Rev. Bras. Ornit. 2007 15, 470-471
Muscicapoidea	Mimidae	Mimus saturninus	Feeding on urban termite and ant swarm; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299; Nuestras Aves 201 63-64
Muscicapoidea	Mimidae	Mimus saturninus	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Muscicapoidea	Mimidae	Mimus macdonaldi	Pecking at bleeding wounds on feet of albatrosses	Condor 1968 70, 269-270
Muscicapoidea	Mimidae	Mimus macdonaldi	Break and feed on eggs of other species	Condor 1968 70, 269-270
Muscicapoidea	Mimidae	Mimus parvulus	Peck at eggs of other birds, eat from eggs broken by other species	Condor 1968 70, 269-270
Muscicapoidea	Turdidae	Catharus frantzii	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Muscicapoidea	Turdidae	Turdus leucomelas	Our record adds a gekkonid species among the lizard prey of this thrush	Rev. Bras. Ornit. 2011 19, 450-452
Muscicapoidea	Turdidae	Turdus leucomelas	Feeding on urban termite swarm; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299
Muscicapoidea	Turdidae	Turdus leucomelas	Prey on small rodent; no record, first report	Rev. Bras. Ornit. 2014 22, 408-410
Muscicapoidea	Turdidae	Turdus flavipes	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Muscicapoidea	Turdidae	Turdus rufiventris	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Muscicapoidea	Turdidae	Turdus rufiventris	Feed on 18cm snake; no reference on vertebrates	Nuestras Aves 1996 33, 31
Muscicapoidea	Turdidae	Turdus subalaris	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Fringillidae	Fringillidae	Carduelis psaltria	Eating soil; only report is on other Carduelis species	Boletín SAO 2006 16, 31-33
Fringillidae	Fringillidae	Euphonia musica	After hurricane, follow bananaquits to take insects under leaves; unusual, undescribed, normally mistletoe gleaner, adaptability, flexibility, plasticity	J. Field. Ornit. 1991 62, 474-478
Fringillidae	Fringillidae	Spinus barbata	Eat seeds of exotic pine; not reported, plasticity	Bol. Chil. Ornit. 2004 10, 18 - 19

Fringillidae	Fringillidae	Loxia megaplaga	Feeding on soils near abandoned bauxite mines; first report	J. Carib. Ornit.2012 25, 98–101
Emberizoidea	Parulidae	Basileuterus culicivorus	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Parulidae	Basileuterus tristriatus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Parulidae	Myioborus miniatus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Parulidae	Myioborus torquatus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Parulidae	Setophaga fusca	Eating Andean Oak catkins; normally eats insects, occasionally fruits, opportunistic exploitation of temporarily superabundant resource	Ornit. Colomb. 2008 78, 78-81
Emberizoidea	Parulidae	Setophaga caerulescens	Feeding on unrefined granulated cane sugar at an outdoor restaurant; adds to the body of knowledge	J. Carib. Ornit.2014 27, 27–30
Emberizoidea	Parulidae	Vermivora chrysoptera	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Icteridae	Cacicus chrysopterus	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Icteridae	Gnorimopsar chopi	First report of predation on a bird (Yellow−billed Cardinal)	Rev. Bras. Ornit. 2008 16, 264-265
Emberizoidea	Icteridae	Gnorimopsar chopi	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Emberizoidea	Icteridae	Icterus icterus	Feeding on wasps outside nests, beating on surface; uncommon, few reports, records which exist are questionable	Ornit. Neotrop. 1997 8, 89-92
Emberizoidea	Icteridae	Molothrus bonariensis	Use twigs to search for seed in bovine feces	Ararajuba 1991 2, 89-90
Emberizoidea	Icteridae	Molothrus bonariensis	Cleaning capybaras; new records	Biota Neotrop. 2010 10, 195-203
Emberizoidea	Icteridae	Molothrus bonariensis	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Emberizoidea	Icteridae	Molothrus bonariensis	Prey on swarming ants; other species mentioned in only other paper	Nuestras Aves 2013 58, 63-64
Emberizoidea	Icteridae	Molothrus oryzivorus	Cleaning capybaras and marsh deer; new records	Biota Neotrop. 2010 10, 195-203
Emberizoidea	Icteridae	Psarocolius montezuma	Predation on young Thraupis episcopus; first report	Zeledonia 2012 16(2), 85-88
Emberizoidea	Icteridae	Pseudoleistes guirahuro	Use twigs to search for seed in bovine feces	Ararajuba 1991 2, 89-90
Emberizoidea	Icteridae	Quiscalus lugubris	Caged adult feeding juvenile outside cage; unusual, one report in other species	El Pitirre 2002 15, 117-120
Emberizoidea	Icteridae	Quiscalus lugubris	Feed on insects under windshield wiper; reported in other species only	El Pitirre 2002 15, 117-120
Emberizoidea	Icteridae	Quiscalus lugubris	Foraging between parked cars in search of food scraps on well-lit parking lot as late as 20,30 in Curacao; opportunistically, not been documented	J Carib Ornithol 2014 27, 40-41

Emberizoidea	Icteridae	Quiscalus niger	Prey on large anole, used technique similar to string pulling; noteworthy	J Carib Ornithol 2006 19, 56-58
Emberizoidea	Thraupidae	Ramphocelus carbo	Feeding on urban termite swarm; ephemeral and unpredictable, departure from usual foraging, first record	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Thraupidae	Tachyphonus coronatus	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Emberizoidea	Thraupidae	Coryphospingus cucullatus	Feed on bamboo seeds; new in the literature	Rev. Bras. Ornit. 2010 18, 344-346
Emberizoidea	Thraupidae	Coryphospingus pileatus	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Emberizoidea	Thraupidae	Sporophila nigricollis	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Emberizoidea	Thraupidae	Sporophila caerulescens	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Emberizoidea	Thraupidae	Coereba flaveola	Eat bread; not reported previously	El Pitirre 2002 15, 117-120
Emberizoidea	Thraupidae	Coereba flaveola	Rob nectar from cordia flowers; no other reports	J. Field. Ornit. 1987 58, 345-349
Emberizoidea	Thraupidae	Coereba flaveola	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Emberizoidea	Thraupidae	Tiaris bicolor	Eat bread; not reported previously	El Pitirre 2002 15, 117-120
Emberizoidea	Thraupidae	Tiaris bicolor	Rob nectar from cordia flowers; no other reports	J. Field. Ornit. 1987 58, 345-349
Emberizoidea	Thraupidae	Loxigilla noctis	Rob nectar from cordia flowers, cuts off corolla, tilts flower up to drink; no other reports	J. Field. Ornit. 1987 58, 345-349
Emberizoidea	Thraupidae	Loxigilla barbadensis	Open sugar packets at localized site; unusual, first recorded observation	J. Field. Ornit. 2002 73, 82–85
Emberizoidea	Thraupidae	Geospiza fuliginosa	Bill shoving search technique, alternative in dry season; unreported	J. Field. Ornit. 1983 54, 421-422
Emberizoidea	Thraupidae	Geospiza fuliginosa	Remove ticks from tortoises who show invitation posture; other artciles mention iguanas	Not. Galap. 1976 24, 4-8;
Emberizoidea	Thraupidae	Geospiza fuliginosa	Drink from pools of blood in afterbirth of sea lions	Condor 1968 70, 269-270
Emberizoidea	Thraupidae	Geospiza fortis	Remove ticks from tortoises who show invitation posture, rarer than in G. fuliginosa; other artciles mention iguanas	Not Galap. 1976 24, 4-8
Emberizoidea	Thraupidae	Geospiza fortis	Bill shoving search technique, alternative in dry season; unreported	J. Field. Ornit. 1983 54, 421-422
Emberizoidea	Thraupidae	Goespiza difficilis	Bill-bracing used to push booby egg on rocks and break them; fascinating, clever	Not Galap. 1983 38, 4-10
Emberizoidea	Thraupidae	Goespiza difficilis	Taking blood from scratch on human, pecking at wound to make it bleed; remarkable and hitherto unrecorded extension	Not Galap. 1984 39, 5
Emberizoidea	Thraupidae	Geospiza conirostris	Ploughing', dig furrows in wet sand to take in water; remarkably resourceful, what other tricks…invented?	Not Galap. 1985 41, 23-24

Emberizoidea	Thraupidae	Camarhynchus pallidus	Using bark or woodchip as a plectrum tool; first time, novel technique	J. Field. Ornit. 1999 70, 104-106
Emberizoidea	Thraupidae	Poospiza lateralis	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Thraupidae	Donacospiza albifrons	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Emberizoidea	Thraupidae	Thlypopsis sordida	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Emberizoidea	Thraupidae	Pyrrhocoma ruficeps	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Thraupidae	Embernagra platensis	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Emberizoidea	Thraupidae	Emberizoides herbicola	Use swarms of army ants to feed on fleeing insects; opportunistic	J. Field. Ornit. 2006. 77, 266–268
Emberizoidea	Thraupidae	Conirostrum cinereum	Secondary nectar robbing; nectar feeding never actually been substantiated	Ornit. Neotrop. 2006 17, 613-617
Emberizoidea	Thraupidae	Conirostrum speciosum	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Emberizoidea	Thraupidae	Pipraeidea melanonota	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Thraupidae	Stephanophorus diadematus	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Thraupidae	Tangara cayana	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Thraupidae	Tangara dowii	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Thraupidae	Pipraeidea bonariensis	Unusual maneuver to reach the sugar water in commercial hummingbird feeders	Atual. Ornit.2007 140, 22
Emberizoidea	Thraupidae	Thraupis sayaca	Intensive folivory; not been reported; normally fruit and insects, unusual	Ararajuba 1998 6, 138-140
Emberizoidea	Thraupidae	Thraupis sayaca	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Emberizoidea	Thraupidae	Thraupis sayaca	Taking small pieces of meat hung in open to dry, ingesting remains of meat adhering to cattle skin drying in sun; surprisingly, typically frugivorous	Lundiana 2005 6, 57-66
Emberizoidea	Thraupidae	Thraupis palmarum	Taking small pieces of meat hung in open to dry, ingesting remains of meat adhering to cattle skin drying in sun; surprisingly, typically frugivorous	Lundiana 2005 6, 57-66
Emberizoidea	Thraupidae	Saltator similis	Feeding on winged termite swarm; unpredictable, never have been seen together, departed markedly from their normal feeding habits	Rev. Bras. Ornit. 2006 14, 297-299
Emberizoidea	Emberiziidae	Chlorospingus flavopectus	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Emberizidae	Melozone leucotis	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275
Emberizoidea	Emberizidae	Pselliophorus tibialis	Feeding on ant swarms; opportunistic, not previously reported	Ornit. Neotrop. 2001 12, 271–275

Emberizoidea	Emberizidae	Zonotrichia capensis	Use lawn mower to feed on disturbed insects; behavioural adjustments, opportunistic	Rev. Bras. Ornit. 2008 16, 387-390
Emberizoidea	Emberizidae	Zonotrichia capensis	Use army ant swarms to feed on fleeing insects; opportunistic, not previously reported, new	J. Field. Ornit. 2009 80, 328–335
Emberizoidea	Emberizidae	Zonotrichia capensis	Feeding on winged termite swarm; opportunity	Lundiana 2003 4, 71
Emberizoidea	Cardinalidae	Cyanoloxia moesta	Feed on bamboo flowers; new in literature	Rev. Bras. Ornit. 2010 18, 344-346
Emberizoidea	Cardinalidae	Piranga flava	First documented case of consuming an anole	Ornit. Colomb. 2003 1, 63-65.
Emberizoidea	Cardinalidae	Rhodothraupis celaeno	Fed on oranges with holes in them; not documented previously	Ornit. Neotrop. 2000 11, 363–364