Zoology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool

Jaw myogenesis in the monk parakeet: evidence of developmental reprogramming in the emergence of novel muscles in Psittaciformes (Aves)

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ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 5 April 2016
Accepted 15 June 2016
Available online xxx

Keywords:
Evo-Devo
Heterochrony
Heterotipy
Morphological novelties
Myiopsitta monachus

ABSTRACT

Psittaciformes have apomorphies in the muscles of the jaw that include both the adductors m. ethmomandibularis (EM) and m. pseudomasseter (PM), which are responsible for the generation of strong bite forces. While the EM is present in all Psittaciformes, the PM can be absent or present, and even underdeveloped or well-developed. The aim of this study is to identify developmental reprogramming processes by comparing the myogenesis of the jaw of the monk parakeet *Myiopsitta monachus* with the information available about other species of Psittaciformes. Seventeen specimens including embryos at different developmental stages, and nestlings of different ages were studied through the analysis of serial histological sections. At embryonic stage 24 (S24) the muscle precursor was observed in the first pharyngeal arch. At S27 the muscle precursor was found to be divided into lateral, intermediate and medial portions. At S31 the independent development of the EM as a rostro-dorsal projection of the mm. pterygoidei could be observed. At S36 the individualization of all muscles was complete. Finally, the PM was detected two days after hatching as an aponeurotic dorsal projection of the m. adductor mandibulae externus superficialis, located lateral to the arcus jugalis. Our results suggest that in *M. monachus* the muscles EM and PM emerge as a result of a process of heterotipy, and variations in the degree of development of the PM are associated to a heterochronic process of post-displacement, with *M. monachus* having an underdeveloped PM with respect to basal Psittaciformes.

1. Introduction

The aim of the evolutionary developmental biology (Evo-Devo) is to identify developmental mechanisms that cause evolutionary changes in phenotypes and the emergence of novel morphological structures (Hall, 2003). It brings together several disciplines such as developmental biology, comparative morphology and evolution (Abdala, 2015). By comparing ontogenetic trajectories, developmental reprogramming processes responsible for the generation of variations can be recognized (Arthur, 2000, 2004). These processes include: heterochrony, which involves changes in time or event rates and/or developmental processes underlying the formation of morphological characters; heterometry, involving a change in the amount of development of a part of an organism with respect to other organisms; heterotopy, which implies that a structure is developed in an unusual position of the body plan; and heterotipy, which is the only process that generates morphological novelties (Arthur, 2000, 2004). Morphological novelties are defined as new structural elements in a body plan which have no homologies in the ancestral organism or even in the same organism (Müller and Wagner, 1991). None of these reprogramming processes occur independently and, in fact, changes taking place during development mostly result due to a concurrence of two or more independent processes (Arthur, 2000, 2004). Regarding heterochronies, Reilly et al. (1997) proposed six processes that result from changes in the rate, the onset and offset of the trajectories of development. These processes include: acceleration and deceleration, by an increase or a decrease of the somatic developmental rate; hypermorphosis and hypomorphosis, involving prolonged or reduced growth; and pre-displacement and post-displacement, by

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http://dx.doi.org/10.1016/j.zool.2016.06.006

Please cite this article in press as: Carril, J., et al., Jaw myogenesis in the monk parakeet: evidence of developmental reprogramming in the emergence of novel muscles in Psittaciformes (Aves). Zoology (2016), http://dx.doi.org/10.1016/j.zool.2016.06.006

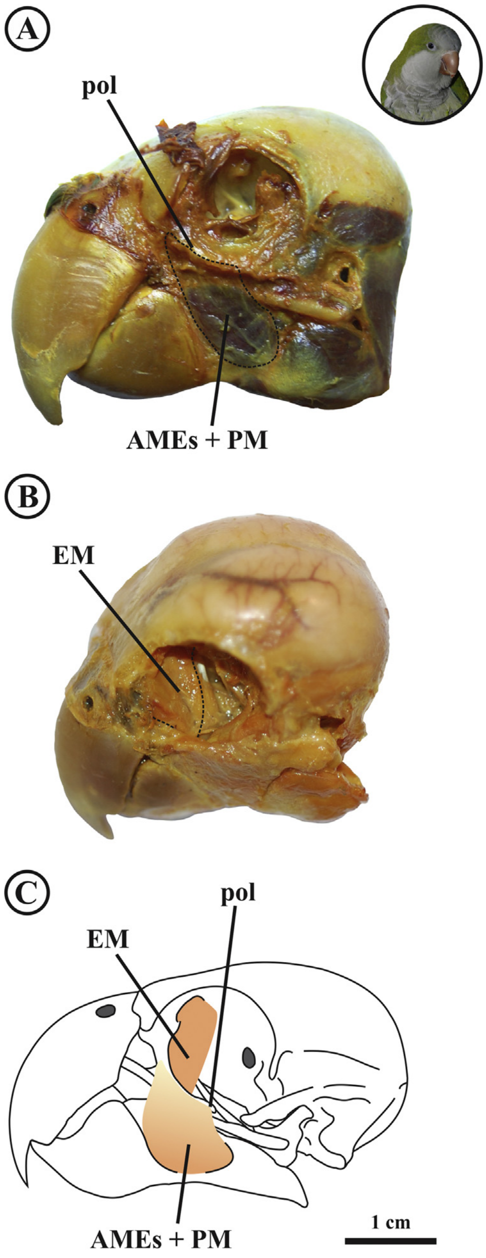

Fig. 1. Myological novelties of the jaw in *Myiopsitta monachus*. (A) Image of a skull showing the m. adductor mandibulae externus superficialis (AMEs) + m. pseudomasseter (PM) and the processus orbitalis of the os lacrimale (pol); (B) similar view showing the m. ethmomandibularis (EM); (C) schematic drawing of the skull showing the novel muscles.

early or late onset of the growth of a structure. These processes can result in paedomorphic (truncated), peramorphic (extended) or isomorphic (equal) features (Reilly et al., 1997).

Psittaciformes are one of the most homogeneous and easily recognizable avian orders (Dyke and Mayr, 1999; Waterhouse, 2006; Forshaw, 2010). They are characterized by advanced cognitive abilities, a highly developed locomotor system and by their special feeding behaviors (Iwaniuk et al., 2005; Carril et al., 2016). Furthermore, their cranio-mandibular complex shows the presence of an ossified arcus suborbitalis (ASO) and the adductors m. ethmomandibularis (EM) and m. pseudomasseter (PM) (Fig. 1), being morphological novelties characteristic of the clade Psittaciformes (Zusi, 1993; Tokita, 2003, 2004; Tokita et al., 2007). Indeed, the ability of Psittaciformes to feed on seeds and nuts is related to the presence of these evolutionary novelties that allow them to exert high bite forces (Burton, 1974; Zusi, 1993; Tokita, 2003, 2004; Bhattacharyya, 2013; Carril et al., 2015).

Table 1
List of embryos (E) and nestlings (N) of *Myiopsitta monachus* used in the present study, indicating the embryonic stage (S) or age in days (d), type of staining performed (HE, haematoxylin–eosin; GT, Gomori trichrome), orientation of sectioning (S, sagittal; C, coronal) and corresponding figures.

Specimen	Stage/Age	Staining	Sectioning	Figures
E133	S23	HE, GT	S	–
E225	S24	HE, GT	S	2A
E259	S25	HE, GT	S	–
E221	S27	HE, GT	S	2B–D
E229	S29	HE, GT	S	–
E192	S31	HE, GT	S	3A
E196	S34	HE, GT	C	3B–C
E219	S34	HE, GT	S	–
E236	S35	HE, GT	C	–
E107	S36	HE, GT	C	3D, 4A
E117	S38	HE, GT	C	4B
E264	S40	HE, GT	S	–
E122	S40+	HE, GT	C	–
N61	0,5 d	HE, GT	C	–
N69	2 d	HE, GT	C	4C
N80*	5 d	–	–	–
N02*	7 d	–	–	4D

* Specimens dissected.

Within Psittaciformes, the presence and/or developmental degree of these myological novelties are variable: while the EM is present in all species of the clade, the PM can be absent or present and if present, in an underdeveloped or well-developed form (Zusi, 1993). Although the study of these morphological novelties would allow a better understanding of their evolutionary origins (Tokita and Nakayama, 2014) and several researchers have investigated the anatomy of the mandibular muscles in adult Psittaciformes (Hofer, 1950, 1953; Porto, 2004; Carril et al., 2014, 2015), only few studies have been dedicated to elucidating the myogenesis of the jaw (Tokita, 2004; Tokita et al., 2007).

The monk parakeet *Myiopsitta monachus* is one of the few Neotropical species among the Psittaciformes for which capture is not restricted due to its status as a pest (Canavelli et al., 2013) and the abundance of specimens in nature. Furthermore, its developmental stages and the adult jaw musculature have been recently studied in detail by our research group, characterizing the presence of the EM and the undeveloped PM (Carril and Tambussi, 2015; Carril et al., 2015). Based on these facts this species represents a good species to study evolutionary reprogramming processes in Psittaciformes. The aim of the present study is to identify developmental reprogramming processes through the comparative analysis of jaw muscle differentiation in the monk parakeet and in other Psittaciformes with various states of morphological novelties.

2. Materials and methods

A total of 17 specimens of *M. monachus* from La Plata (Buenos Aires) and Dean Funes (Córdoba), Argentina, were obtained from nests during breeding seasons from 2011 to 2013, sacrificed according to protocols approved by the animal care committee and adhering to the legal requirements of Argentina, fixed by immersion in a 4% formaldehyde solution for 48 h and preserved in 70% alcohol (Table 1). The assignment of embryonic stages (23–40+) was performed based on descriptions by Carril and Tambussi (2015) and the nestlings' age (from newly hatched to 7 days old) was calculated using the length of the hindlimbs' digit III as proposed by Aramburú (1997). Using standard histological techniques, serial sections (3 μm) parallel or transverse to the sagittal plane were prepared. The material was stained with haematoxylin–eosin for general histological description and with Gomori trichrome for a

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