



## Geometric morphometrics of the skull of Tinamidae (Aves, Palaeognathae)

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### ABSTRACT

The Tinamidae comprise exclusively Neotropical palaeognathous birds, with homogeneous body morphology and no sexual dimorphism. The goal of this work was to explore the variation in skull morphology between taxa and its possible correspondence with features such as diet or gender using geometric morphometric tools. Eleven landmarks were analyzed in 53 skulls of 4 genera that inhabit grasslands: *Nothoprocta*, *Eudromia*, *Nothura* and *Rhynchotus*. Intrageneric and intergeneric variability was analyzed. The genera studied here can be distinguished based on the geometric shape of their skull, with preauricular region length and neurocranium shape as the most outstanding features. In the genus *Eudromia*, males and females could be differentiated, while in the genus *Nothoprocta*, the species differentiated according to their trophic habits. This study allows establishing that genera and, in some cases, the gender of the Tinamidae can be differentiated based on cranial shape.

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### 1. Introduction

The family Tinamidae (Aves, Palaeognathae) comprises 47 species grouped in 9 genera, distributed in the Neotropical region from Northwest Mexico to southern South America (Cabot, 1992). These birds with poor flight capability occupy diverse environments including Altoandean steppes (e.g., puna tinamou *Tinamotis pentlandii*), low grasslands (e.g., spotted nothura *Nothura maculosa*) and rainforests (e.g., solitary tinamou *Tinamus solitarius*). Except for early anatomical studies (e.g., Parker, 1862; Alix, 1874; Lucas, 1886; Pycraft, 1900), the skull anatomy of the Tinamidae has received little attention. The most recent contributions are those of Silveira and Höfling (2007) and some studies related to fossil Tinamidae (e.g., Tambussi, 1987; Bertelli and Chiappe, 2005; Picasso and Degrange, 2009).

Traditionally, the characterization of tinamid taxonomic groupings at all levels has been almost completely based on integumentary characters (Bertelli et al., 2002), while osseous features were neglected, except for Bertelli and Porzecanski (2004) who combined morphology with molecular data (Fig. 1).

Specifically, the cranial anatomy of these birds was studied and compared in detail only by Silveira and Höfling (2007). These authors concluded that skull anatomy provided accurate information for the recognition of two groups: Tinaminae and

Nothurinae, which was in accordance with previous studies (e.g., Miranda-Ribeiro, 1938). Nevertheless, these authors did not make an exhaustive description and differentiation of the osseous features that characterize each genus.

The goals of this study were: (i) to find patterns of differentiation in the cranial shape of eight species belonging to four genera present in Argentina using a novel tool, geometric morphometry and (ii) to explore whether the patterns of skull shape variation can be related to gender or feeding habits.

### 2. Materials and methods

In this analysis, 53 skulls of 8 species belonging to 4 of the 7 genera present in Argentina (Fig. 1) were used. These are diurnal species that occupy mainly grasslands: *Nothoprocta* (Andean tinamou *N. pentlandii*,  $n=4$ ; brushland tinamou *N. cinerascens*,  $n=8$ ; ornate tinamou *N. ornata*,  $n=1$ ), *Eudromia* (elegant crested tinamou *E. elegans*,  $n=11$ ; Quebracho crested tinamou *E. formosa*,  $n=1$ ), *Nothura* (Darwin's nothura *N. darwini*,  $n=8$ ; spotted nothura *N. maculosa*,  $n=12$ ) and *Rhynchotus* (red-winged tinamou *R. rufescens*,  $n=8$ ). More information about the investigated specimens is available as electronic supplement at doi:10.1016/j.zool.2010.07.003.

The skulls are housed in the collections of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Ciudad Autónoma de Buenos Aires, Buenos Aires province, Argentina (MACN) and the Museo de La Plata, La Plata, Buenos Aires province, Argentina (MLP). Osteological terminology follows Baumel and Witmer (1993).

Each skull was photographed in dorsal view with the same focal length following the guidelines presented by Zelditch et al. (2004), using a digital camera (DSC H5; Sony Corp., Tokyo, Japan). This view

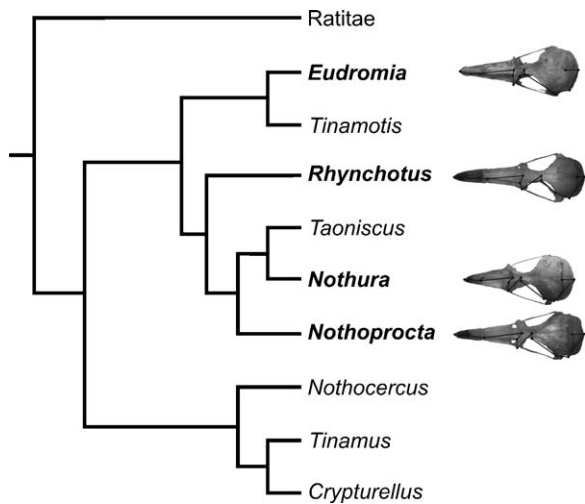
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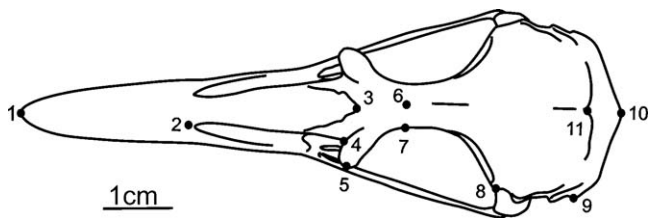
(F.J. Degrange).

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**Fig. 1.** Simplified cladogram modified from Bertelli and Porzecanski (2004) showing the phylogenetic relationships of the four genera studied here (in bold) as well as their respective cranial morphologies.



**Fig. 2.** Landmarks used in this analysis shown on the left side of a tinamid skull, dorsal view.

was chosen for best visualization of the shape of the rostrum, os lacrimale and crista nuchalis.

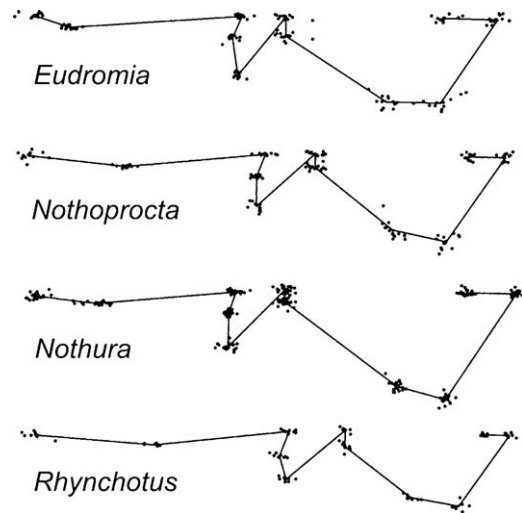
Eleven landmarks were selected (Fig. 2), corresponding to the tip of the bill (landmark 1), the posterior region of the bill (landmarks 2–5), the interorbital region (landmarks 6 and 7), the temporal region (landmarks 8 and 9) and the parietal region (landmarks 10 and 11). The x and y coordinates of each landmark were digitized using the software TpsDig 1.41 (Rohlf, 2005).

The resulting coordinates were subjected to a generalized Procrustes analysis, which removes all the information unrelated to shape (Rohlf and Slice, 1990; Zelditch et al., 2004). Localized shape changes were estimated by partial warp scores (Bookstein, 1991). Once these values were obtained, a relative warps analysis (RWA; Rohlf, 1993), i.e. a principal components analysis of the partial warp scores, was performed to examine skull shape changes. The RWA was performed using the software TpsRelw 1.35 (Rohlf, 2003) and IMP (Sheets, 2003).

**Table 1**

Overview of the main cranial features, diet and intrageneric differences in the four genera studied.

Genera	Main cranial features	Diet	Intrageneric differences
<i>Eudromia</i>	Short beak, short prenarial region, long and narrow neurocranium	<i>E. elegans</i> : herbivorous and insectivorous <i>E. formosa</i> : no information about feeding habits	<i>E. elegans</i> : males and females with differences in the parieto-temporal region
<i>Nothoprocta</i>	Long bill, long prenarial region, wide and short neurocranium	<i>N. cinerascens</i> : mainly animalivorous <i>N. pentlandii</i> and <i>N. ornata</i> : herbivorous and insectivorous	<i>N. pentlandii</i> , <i>N. ornata</i> , and <i>N. cinerascens</i> : differences in the parieto-temporal region
<i>Nothura</i>	Short beak, short prenarial region, wide and long neurocranium	<i>N. darwinii</i> : insects and especially seeds <i>N. maculosa</i> : prefers plant material	<i>N. darwinii</i> and <i>N. maculosa</i> : differences in the posterior region of the beak and the prenarial region
<i>Rhynchotus</i>	Very long beak, long prenarial region, short and narrow neurocranium	Herbivorous and insectivorous	<i>R. rufescens</i> : very little variation



**Fig. 3.** Intrageneric skull variation.

The relative warps (RW) obtained were used to analyze whether the morphological groups were consistent with the diet and gender of the specimens. Data on diet for each species was taken from Davies (2002). The initial analysis considered the variability between species belonging to different genera (i.e., intergeneric analysis); subsequently, the variability within each genus (i.e., intrageneric analysis, Fig. 3) was also analyzed. To assess differences in skull shape between genera, a MANOVA was performed using genus as the independent variable and the partial warp and uniform component scores as dependent variables.

**3. Results**

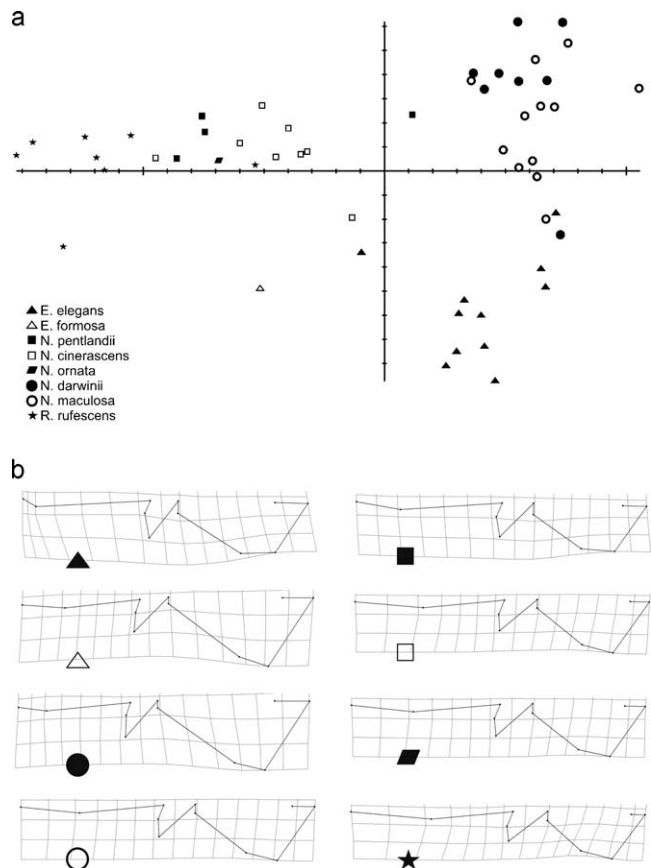
Table 1 summarizes the main cranial features and the diet of each genus and species analyzed here.

**3.1. Intergeneric analysis**

The MANOVA test for significant differences in skull morphology between genera was significant (Wilks'  $\Lambda = 0.0149$ ,  $F_{(96,16)} = 5.525$ ,  $p = 1.923 \times 10^{-13}$ ).

Two analyses were conducted. The first one included all the specimens and it showed partial segregation between genera; the separation was not complete due to some specimens that presented a problematic shape (Fig. 4).

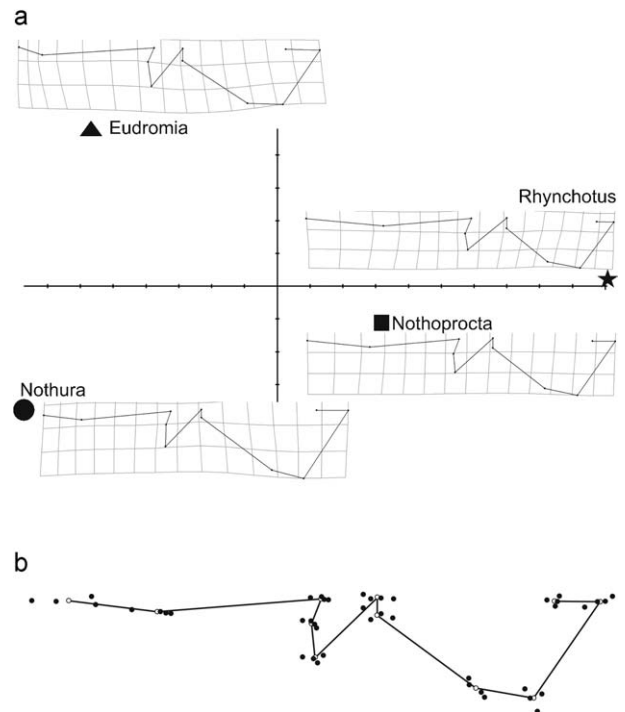
The first and second RW explained 63.83% and 17.43% of the variation, respectively. Apart from the conflicting specimens, generally speaking the left upper quadrant was occupied by the genera *Rhynchotus* and *Nothoprocta*. Their skulls are characterized by long bills



**Fig. 4.** (a) Relative warp analysis of the 8 tinamid species analyzed. Horizontal axis corresponds to RW1, vertical axis corresponds to RW2. (b) Grids for visualization of the shape changes in each species.

with a long pre-narial region (located between landmarks 1 and 2, Fig. 2), an anteromedially directed temporal region, and a short, broad neurocranium. *Nothura* is located in the right upper quadrant, which corresponds to forms characterized by short bills with a short pre-narial region, an anteromedially directed temporal region and a very wide, long neurocranium. The right lower quadrant is mainly occupied by *E. elegans*, characterized by a short bill with a short pre-narial region, a temporal region parallel to the sagittal plane and a long, narrow neurocranium. The left lower quadrant is occupied by *Eudromia*, *Rhynchotus* and *Nothoprocta* specimens that show a long bill with a medium-length pre-narial region, a temporal region parallel to the sagittal plane and a short, narrow neurocranium (Fig. 4).

In the second analysis, the consensus individual for each genus was obtained and these were compared with each other (Fig. 5a). The first two RW explained 83.38% and 15.92% of the variation, respectively. The results show that the tip of the bill, the interorbital region and the anterior portion of the neurocranium (landmarks 1 and 6–10) are the most variable, while the basal portion of the bill and the posterior neurocranium (landmarks 2–5 and 11) are more conservative (Fig. 5b). These results suggest several morphological skull types among the tinamids. The *Eudromia* skull morphotype has a short bill with a short pre-narial region, a long and narrow neurocranium and a large orbit (distance between landmarks 5 and 8, Fig. 2). The *Nothura* morphotype has a short bill with a short pre-narial region, a wide and long neurocranium and a very large orbit (the largest among the genera studied here). The *Nothoprocta* morphotype shows a long bill with a long pre-narial region, a wide but short neurocranium and a large orbit. The *Rhynchotus* morphotype has a very long beak (much longer than in the other genera) with a



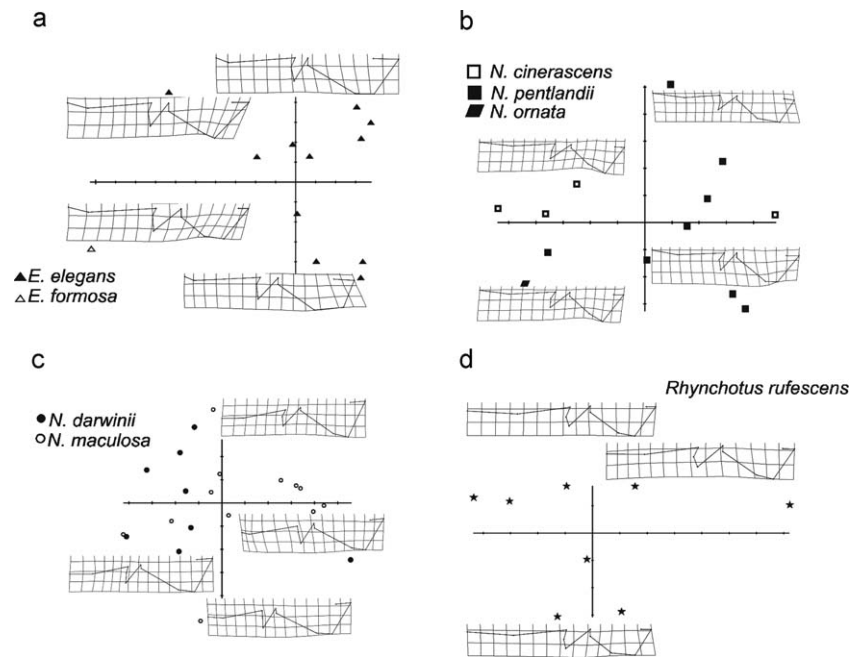
**Fig. 5.** (a) Relative warp analysis of the consensus shapes for each genera. Grids are included for visualization of shape changes in each genus. Vertical axis corresponds to RW1, horizontal axis corresponds to RW2. (b) Skull variation in the four genera studied here. Empty circles indicate the consensus individual.

long pre-narial region, a short and narrow neurocranium and a small orbit. *Eudromia* is the only genus with its parieto-temporal region parallel to the sagittal plane, whereas in the other genera this region is anteromedially slanted. The orbit presents a slight craniomesial orientation in all the genera, more marked in *Nothura*.

### 3.2. Intrageneric analysis

In *Eudromia*, the parieto-temporal region was the most variable while the bill was the most conservative. The single specimen of *E. formosa* was clearly separated from *E. elegans* in the analysis. The first two RW explained 47.39% and 27.93% of the total variance, respectively (Fig. 6a). Even though *E. elegans* showed some differences in the temporal region of the neurocranium, they did not show any gender-related segregation in the plot. However, the mean shapes of males and females were different (Goodall test,  $F=3.81$ ;  $df=18,14$ ;  $p=2.82 \times 10^{-6}$ ). The parieto-temporal region of males was shorter and parallel to the sagittal plane, whereas in females this region was longer and oblique.

In *Nothoprocta*, the greatest variation occurred in the tip of the bill and the temporal region, while the posterior region of the bill and the parietal region were less variable. RWA allowed discriminating the herbivorous species *N. pentlandii* and *N. ornata* from the animalivorous species *N. cinerascens* by shape differences in the neurocranium, mainly in the parieto-temporal region (Fig. 6b). The first two RW explained 43.3% and 17.83% of the variation, respectively. The mean shapes of *N. cinerascens* and *N. pentlandii* were statistically different (Goodall test,  $F=2.40$ ,  $df=18,18$ ;  $p=0.001$ ). In *N. cinerascens*, the temporal region was anteromedially directed; in *N. ornata*, this region was strongly anteromedially directed, while in *N. pentlandii* it was parallel or subparallel to the sagittal plane. The three species had a long bill with an elongated pre-narial region. The neurocranium was broad in the three species, being broadest in *N. ornata* and longest in *N. pentlandii*.



**Fig. 6.** Relative warp analysis. Grids are included for visualization of shape changes in each quadrant. (a) *Eudromia* species, (b) *Nothoprocta* species, (c) *Nothura* species, (d) *Rhynchotus* specimens. In each scatterplot, the vertical axis corresponds to RW1, the horizontal axis corresponds to RW2.

In *Nothura* (Fig. 6c), the anterior region of the bill and the interorbital region proved to be the most variable while the posterior region of the bill and the parietal region were the most conservative. When *N. maculosa* and *N. darwinii* were plotted together, it was not possible to separate any group or make any accurate association of shape with the analyzed variables. The first RW explained 41.46% of the total variation while the second explained 20.99%. Statistically, the mean shapes of these two species were significantly different (Goodall test,  $F = 2.44$ ,  $df = 18,324$ ,  $p = 0.001$ ). In *N. darwinii*, the posterior region of the bill was narrower and the pre-narial region was much longer than in *N. maculosa*.

The skulls assigned to *R. rufescens* (Fig. 6d) showed very little variation. The first two RW explained 60.19% and 19.96% of the variation, respectively.

#### 4. Discussion

The four genera studied here can be characterized and differentiated on the basis of the geometric shape of their skulls. The basic features for discrimination are bill length, length of the pre-narial region, and width and length of the neurocranium. Due to the paucity of sexed material, it was not possible to differentiate males from females at the intrageneric level, with the exception of *E. elegans*. The analysis clearly separated the only available specimen of *E. formosa* from the specimens of *E. elegans*. These two species live in different environments, in Chacoan environments and shrub steppes, respectively (Davies, 2002). In the species of *Nothura* and *Nothoprocta* (excluding *N. ornata* due to the availability of a single individual only), there were differences between the mean skull shapes of *N. cinerascens* and *N. pentlandii* on the one hand and *N. maculosa* and *N. darwinii* on the other hand. In other words, these species are distinguishable at a taxonomic level through an analysis of this kind. In the case of *N. pentlandii* and *N. cinerascens*, the cranial morphological differences may reflect differences in their diet. The only species of the genus *Rhynchotus* studied here appeared to be conservative in its cranial morphology.

Theoretically, species that occupy homogeneous habitats such as the ones studied here, are precluded from partitioning food resources by using different microhabitats (Barbosa and Moreno,

1999). In certain regions of Argentina, two or more species can cohabitate, even congeneric ones (Cabot, 1992; Davies, 2002; Narosky and Yzurieta, 2003). Tinamous show two mechanisms of avoiding competition (Cabot, 1992): (i) an extremely precise habitat selection which enables species to inhabit different areas of the same habitat where variation is barely perceptible yet sufficient to avoid interference and (ii) differences in body size, leading to a preferential selection of food (Cabot, 1992). In this context it can be assumed that this partition implies differences in foraging strategies and such differences would be reflected in morphological differences (i.e., cranial disparity). For the taxa studied here, both the bill (which captures the food) and the neurocranium (where the jaw muscles which handle the food are attached) are highly variable. The variation found in the osseous anatomy of these skull regions might be related to the feeding mechanism, which is in turn linked to the degree of development of the jaw muscles. However, since these anatomical variations occur both between and within the genera (see the MANOVA), no morpho-functional correlation seems to exist between feeding habits and skull shape. The Tinamidae probably had an evolutionary constraint that prevented any dietary specialization, a fact also reflected in their morphology, physiology and behavior (Zweers et al., 1997; Garitano-Zavala et al., 2003). This constraint could be due to the mixed diet shared by all tinamous (with some seasonal variations) that includes both animals (particularly invertebrates) and vegetable matter. Nevertheless, some species tend to be more herbivorous while others are more carnivorous (Cabot, 1992). This could be the case with the species of genus *Nothoprocta* (Fig. 6b) in which the herbivorous *N. pentlandii* was separated from the mainly carnivorous *N. cinerascens*.

Geometrical morphometric analyses of avian anatomy are rare, and studies relating anatomical variations to ecological variables are even more scarce. Acosta Hospitaleche and Tambussi (2006) studied the skull of *Pygoscelis* (Sphenisciformes) and found only small differences between the three living species of the genus. In this case it was not possible to distinguish the effect of gender on intraspecific variation. Acosta Hospitaleche (2009) also studied another genus of Sphenisciformes, *Spheniscus*. In this case, the extension of the *fossa temporalis* was found to be the most variable

skull feature. The main jaw muscle, the *m. adductor mandibulae externus*, originates from this fossa and is related to the predatory activity of these birds. Finally, in a study of two species of *Stercorarius* (Charadriiformes), Acosta Hospitaleche et al. (2009) found no differences due to taxonomy, gender or geographical distribution through similar analyses.

This is the first study to explore the skull shape of tinamids through geometric morphometric techniques. The research conducted here allows establishing that, although the tinamids present a homogeneous external morphology without sexual dimorphism (Cabot, 1992), the genera and, in some cases, the gender can be differentiated based on their cranial shape. It is to be hoped that a comparison that comprises more species (including those forms that inhabit forest environments, i.e. the Tinaminae) may provide important information on their food selection, distribution, sexual dimorphism and phylogeny.

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### Appendix A. Supplementary data

Additional information associated with this article can be found in the online version at doi:10.1016/j.zool.2010.07.003.

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