AMPHIBIAN ASYMMETRY, A USEFUL TOOL TO ASSESS ENVIRONMENTAL QUALITY

L´ASYMÉTRIE DES AMPHIBIENS, UNE MÉTHODE UTILE POUR ÉVALUER LA QUALITÉ DE L´ENVIRONMENT

L´ASIMMETRIA DEGLI ANFIBI, UN METODO UTILE PER VALUTARE LA QUALITÀ AMBIENTALE

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Abstract

This study aimed to determine the morphological stress experienced by *Rana perezi*, an anuran endemic to the Iberian Peninsula, in an agricultural landscape with different levels of management impact, by using the fluctuating asymmetry (FA) of its skeleton. Tibio-fibula, a highly functional trait, had the lowest FA level, whereas radio-ulna was the most asymmetric. This indicates that traits under directional selection are more prone to reflect stress than the functionally important ones. Metatarsus and radio-ulna were less able to cope with the stress in rice fields than in reservoirs. In ontogeny FA level decreased towards the adult stage suggesting that the mechanism of developmental stability buffered against the stress. In conclusion metatarsal and radio-ulna asymmetry of *R. perezi* juveniles can be used to assess environmental disturbances in agricultural landscapes.
Keywords: *Rana perezi*; fluctuating asymmetry; agricultural landscape; environmental stress.

Résumé

Cette étude a visé de déterminer le stress morphologique éprouvé par *Rana perezi*, un anoure endémique de la Péninsule Ibérique, dans le paysage agricole, en utilisant l'asymétrie fluctuante (AF) de son squelette. Le tibio-péroné, un trait très fonctionnel, montrait le plus faible degré d'asymétrie, alors que le radio-cubitus a était le plus asymétrique. Il révèle que les traits sous sélection directionnelle sont plus prédisposés au stress que les traits fonctionnels importants. En outre, le métatarse et le radio-cubitus font moins face aux stress dans les rizières que dans les réservoirs. Dans l'ontogenèse, le niveau d'AF a diminué en s'approchant du stade, suggérant que le mécanisme de la stabilité développementale protège contre le stress. En conclusion, l´AF du métatarse et du radio-cubitus des juvéniles de *R. perezi* pourrait être utilisée pour l'évaluation des perturbations environnementales dans des paysages agricoles.

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Mots-clés : *Rana perezi;* asymétrie fluctuante; paysage agricole; stress environnemental.

Riassunto

Questo studio mira a determinare lo stress morfologico vissuto da *Rana perezi*, un anura endemico della Penisola Iberica, nel paesaggio agricolo, utilizzando l'asimmetria fluttuante (AF) del suo scheletro. Il tibio-perone, un tratto altamente funzionale, ha mostrato il più basso grado di AF, mentre la radio-ulna è risultata la più asimmetrica. Si rileva che tratti sotto selezione direzionale sono più inclini a riflettere stress che tratti funzionalmente importanti. Inoltre, metatarso e radio-ulna sono meno capaci di far fronte allo stress in campi di riso che in riserve d'acqua. In ontogenesi, il livello di AF diminuisce verso la fase adulta suggerendo che il raggiungimento di stabilità nello sviluppo dell'organismo rappresenta una protezione dallo stress. In conclusione l´asimmetria del metatarso e radio-ulna di forme giovani di *R. perezi* può essere utilizzato per valutare disturbi ambientali in paesaggi agricoli.
Parole chiave: *Rana perezi;* asimmetria fluttuante; paesaggio agricolo; stress ambientale.

Introduction

Biotic indexes and physiochemical parameters have been largely used for the assessment of environmental quality. Environmental agencies are, however, constantly looking for economic and efficient alternatives which can effectively measure and monitor the condition, integrity and long-term sustainability of ecosystems. A way to examine the environment stress in organisms, before their decline or extinction occurs is to study their fluctuating asymmetry (FA: small random deviations from perfect symmetry, Møller & Swaddle 1997) and therefore indirectly evaluate the environmental quality. Amphibians are one of the nature´s best indicators of overall environmental health as they inhabit two niches (aquatic and terrestrial) and are sensitive to local factors such as water quality and microhabitat availability (Pope 2000). Agricultural management of wetlands is one of the major stressors of amphibian populations (Piha 2006). Reduced survivorship, altered feeding activity and mobility, decreased growth, as well as morphological deformities and increased asymmetry have been reported as main effects of pesticides use on amphibians (Söderman *et al.* 2007; Piha *et al*. 2006).
The objective of the present study was to determine the fluctuating asymmetry of a number of elements of *R. perezi* skeleton in two areas with different levels of agricultural impact: intense and low management activity.

Materials and Methods

Monegros (NE Spain) is one of the most arid regions of Europe (Herrero & Snyder 1997). The area has been identified as being particularly vulnerable to human and climate-induced land degradation (Macklin *et al.* 1994). *R. perezi* is a permanent

resident of Monegros irrigated area. A number of 562 individuals were captured randomly in rice fields and their supplying reservoirs, during the dry (January-May) and wet period (July-September) of 2003. The samplings took place in 5 municipalities of Monegros: San Juan del Flumen, Cartuja, Lanaja, Orillena and Sariñena. The frogs were classified in sex - size classes and X-rayed. Thereupon they were back released in the environment. Three traits were measured repeatedly by image analysis: radio-ulna, tibio-fibula and the second metatarsal bone.

The absolute values of the difference (R-L) of metatarsus showed significant correlation with trait size and frog size. This was corrected using the expression FA=|R-L|/ [(R+L)/2] (Palmer & Strobeck, 1986). In addition, we tested for directional asymmetry by comparing unsigned FA values *vs.* 0 with *t* tests, and for antisymmetry with Kolmogorov- Smirnov tests.

ANCOVA helped testing FA differences between landscape type and period (dry/wet). Descriptive statistics are given ±SE.

Results

Measurement error for all traits was smaller than the FA values which indicated true asymmetry. This followed a normal distribution with mean zero for all traits considered.

The FA of the 3 studied bones showed significant differences between frog classes. The most affected by stress was radio-ulna (FA=0.71±0.02), followed by metatarsal (0.56±0.02) and tibio-fibula (0.21±0.007). Along the postmetamorphic development FA decreased from recently metamorphosed (n=148) and juveniles (n=147) to adult males (n=98) and females (n=169) (Fig. 1).

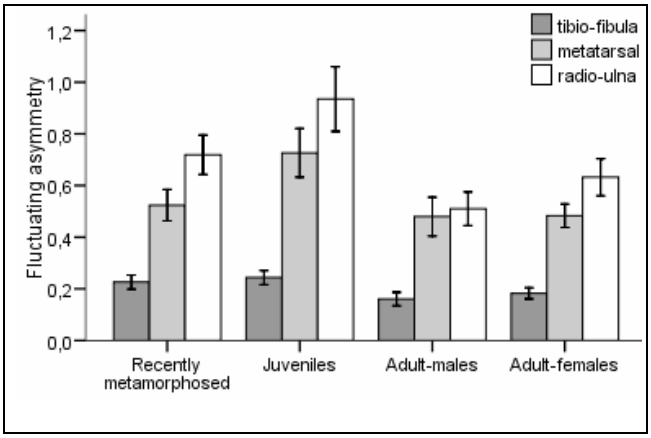

Figure 1

Fore and hind-limbs FA (adjusted for trait size) in ontogeny.
Values plotted are means ±SE.
FA = |R-L|/[(R+L)/2], where R=length of the right limb, L=length of the left limb and size of the limb = (R+L)/2.

A one way ANOVA showed significant differences of juveniles´ radio-ulna FA ($F=8.90$, $P<0.05$) as well as of metatarsal and radio-ulna of adult males ($F=6.21$ and $F=13.59$, respectively, $P<0.05$) between rice fields and reservoirs. As expected FA values were higher in rice fields than in reservoirs especially in the dry period (Fig. 2).

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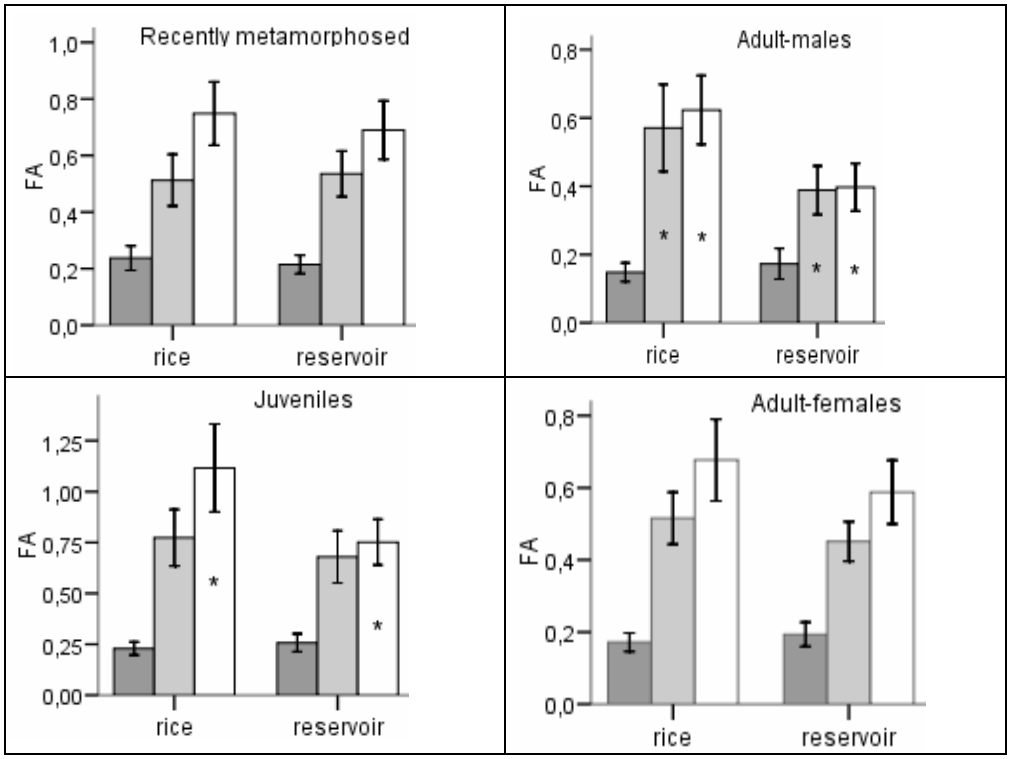

Figure 2 - *Fluctuating asymmetry (FA) of tibio-fibula ( ), metatarsal ( ) and radio-ulna ( ) in rice fields and reservoirs: (a) recently metamorphosed, (b) juveniles, (c) adult-males and (d) adult-females. *Significant differences of FA between rice fields and reservoirs, P<0.05.*

The analysis of covariance (ANCOVA) revealed that the combination landscape type-dry/wet period had a strong influence on the level of metatarsal and radio-ulna asymmetry (F=6.46 and F=8.26, respectively; P<0.05). Accordingly, in rice fields the values of metatarsal FA were significantly higher in the dry period (FA=0.64±0.04) than in the wet period (FA=0.51±0.04) (ANCOVA F=11.11, P<0.01); radio-ulna asymmetry followed a similar trend: in dry period FA=0.86±0.05; in irrigated period FA=0.70±0.05 (ANCOVA F=13.12, P<0.01).

Discussion and Conclusions

The results revealed that tibio-fibula, a highly functional trait, exhibited the lowest level of asymmetry, illustrating thus its ability to cope with stress during development. Generally, morphological features of hindlimbs are important determinants of locomotory performance in amphibians (Martín & López 2001). The highest level of FA was registered by a forelimb trait (i.e. radio-ulna), which is used by *R. perezi* males to hold females during copulation. It thus confirms the

hypotheses that traits under directional selection are more sensitive to stress (Møller & Pomiankowski 1993).
In ontogeny decreasing levels of FA towards the adult stage illustrates that developmental stability at earlier stage is efficient at buffering against the stress. The higher degree of FA in the smaller sex (i.e. females) suggests that the sex difference in FA is indeed related to sex per se, rather than to the degree of sexual size dimorphism (Söderman *et al.* 2007).
Our study established a significant connection of metatarsal and radio-ulna FA with the landscape management level suggesting that the intensive agricultural practices could result in development instability. It seems that less-functional skeleton traits of *R. perezi* can be used to estimate the level of environmental disturbance. Further studies are required to establish which agricultural practices and environmental parameters are more involved in determining the morphological asymmetries of *R. perezi*.
In the general context of world amphibian decline (Alford & Richards 1999) FA may well reflect populations under stress and indirectly, the environmental quality.

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