

A NON-INVASIVE GENETIC SURVEY OF THE PINE MARTEN (*MARTES MARTES*) IN THE WESTERN RIVER PO PLAIN (ITALY): PRELIMINARY RESULTS

ALESSANDRO BALESTRIERI¹, ARITZ RUIZ-GONZÁLEZ², LUIGI REMONTI¹, BENJAMÍN J. GÓMEZ-MOLINER², SARA GENOVESE¹, LAURA GOLA³, CLAUDIO PRIGIONI^{1*}

¹Dipartimento di Biologia Animale, Università di Pavia, Piazza Botta, 9 27100 Pavia;

*Corresponding author, e-mail: prigioni@unipv.it

²Department of Zoology and Animal Cell Biology, Zoology Laboratory, Facultad de Farmacia, Universidad del País Vasco (UPV-EHU), C/ Paseo de la Universidad 7 01006 Vitoria, Spain

³Parco Fluviale del Po e dell'Orba, tratto Vercellese-Alessandrino, Piazza Giovanni XXIII 6, 15048 Valenza (AL)

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RIASSUNTO - *Monitoraggio genetico non invasivo della martora (*Martes martes*) nella pianura padana occidentale: risultati preliminari.* Tra il 2003 e il 2004, due esemplari di martora (*Martes martes*) sono stati investiti da automezzi sulla sponda sinistra del fiume Po (R. N. Garzaia di Valenza, Piemonte). Per accertare la presenza stabile della specie nella Riserva e lungo il tratto vercellese-alessandrino del Parco fluviale del Po, è stato applicato un metodo PCR-RFLP per l'analisi di mtDNA di origine fecale. A partire da gennaio 2007, sono state raccolte 212 feci attribuibili al genere *Martes* in base a forma e dimensioni. L'analisi di un campione di 117 feci (55%) ha permesso di identificare 10 feci di martora e 15 di faina (*M. foina*), confermando la presenza della martora nella Riserva e accertandola per la prima volta in un'area collinare in sponda destra del Po (Camino, AL), circa 32 km a monte della Riserva. Resta da valutare se la scarsa resa dell'amplificazione del DNA (21,4%) sia dovuta alla degradazione del materiale genetico o ad un numero elevato di feci di volpe (*Vulpes vulpes*) erroneamente attribuite a *Martes* durante la fase di raccolta.

Parole chiave: PCR-RFLP, *Martes foina*, species identification, faecal DNA

The Italian range of the pine marten (*Martes martes*) includes the Alps, the Apennines, Sardinia, Sicily and the Isle of Elba, but little is known about numbers and trend of its populations (Genovesi and De Marinis, 2003).

The pine marten is believed to occur primarily in well structured forest habitats (Buskirk, 1992), but in southern Europe it has shown great ecological plasticity, having been reported in coppices, the Mediter-

ranean maquis and cultivated land with woodland fragments (De Marinis and Masseti, 1993; Pittiglio, 1996). In northern Italy, the pine marten is mainly associated with deciduous and coniferous forests between 1000 and 2000 m a.s.l. (Martinoli, 2001), but in the last ten years it has been reported also for several localities of the western River Po plain (NW Italy), where road kills have highlighted the presence of this mustelid in both arable land and urban

surroundings. The penetration of the pine marten into agricultural areas probably follows the main watercourses, which still offer patches of semi-natural woodland (Balestrieri *et al.*, 2008).

Road kills are unpredictable events which, if regularly filed over long periods, can help to draw a large-scale picture of species distribution, but are ineffective when trying to examine their actual range and habitat preferences at a local scale. On the contrary, the systematic survey of field signs, mainly faeces, can yield reliable results about the distribution, abundance and habitat requirements of carnivores (Sadlier *et al.*, 2004).

The pine marten lives sympatrically across a large part of Europe with the closely related stone marten (*Martes foina*), which is quite similar in terms of morphology and feeding habits (Marchesi *et al.*, 1989).

The monitoring of European pine marten populations is hindered by our inability to distinguish the faeces from the two species, making indirect survey methods unreliable. Moreover martens' faeces can be confused with those of other carnivores, such as, for northern Italy, the red fox *Vulpes vulpes* and the polecat *Mustela putorius* (Davison *et al.*, 2002).

Nonetheless, recent progress in molecular techniques has supplied several non-invasive genetic methods for species identification (review in Ruiz-González *et al.*, 2008) which can be applied to field monitoring.

In February 2003 and March 2004, two pine martens were killed by cars inside the Natural Reserve "Garzaia di Valenza", a 12.3 km² wide protected area on the left bank of the River Po, included in the River Po Park (SE Piedmont, NW Italy). The whole territory is flat, mainly cultivated. Small wood patches border an abandoned river meander and three naturalized artificial lakes.

To determine the stable presence of the spe-

cies in the Reserve and investigate its distribution along the western River Po plain we applied a polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method specifically designed for distinguishing between *M. martes* and *M. foina* by the analysis of faecal mitochondrial deoxyribonucleic acid (mtDNA; Ruiz-González *et al.*, 2008).

Since January 2007, a total of 212 "marten-like" faeces has been collected through weekly surveys along fixed transects. During the first three months, eight areas were surveyed (Fig. 1); successively, the monitoring was limited to the two areas regularly providing some faecal samples, the above cited Reserve and a hilly area on the right bank of the river, some 32 km upstream (included between the towns of Camino and Pontestura, Alessandria province).

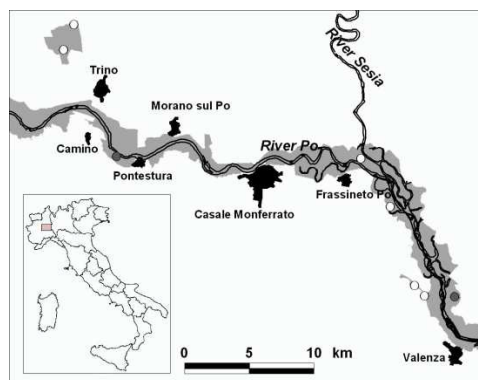


Figure 1 - Study area (River Po Park) with the eight sampling stations (grey dots: positive stations).

Faecal samples were initially assigned to the genus *Martes* if less than 10 mm large and to the red fox when larger than 15 mm. Samples with intermediate width were attributed on the basis of their overall appearance.

A portion (about 30%) of each "marten-like" faecal sample was picked up with

sticks and preserved in 96% ethanol and by freezing until DNA extraction, the rest was retained for dietary analysis. The UTM coordinates of each sample were filed and projected onto a GIS (Arcview 3.1 ESRI). The faecal mtDNA extraction procedure was based on the protocol described by Gómez-Moliner *et al.* (2004). Two specifically designed primers were used, which generate 276 bp long amplicons. These primers amplify the DNA from the two *Martes* species and from the other four *Mustela* species, whilst the DNA from foxes or martens' prey render no amplicons (except for 900 bp long amplicons from *Sorex* spp.). The simultaneous use of the restriction enzymes *RsaI* and *HaeIII* differentiated *M. martes* from *M. foina* and them both from the other carnivore species whose DNA is amplified by the selected primers (see Ruiz-González *et al.*, 2008 for further details).

The genetic analysis of a sub-sample of 117 faeces (55%) has yielded 25 identifications, corresponding to 10 *M. martes* scats and 15 *M. foina* scats. The presence of the pine marten has been confirmed in the Reserve and ascertained for the first time in the Camino-Pontestura area.

The rate of success of DNA amplification was 21.4%, a much lower rate than that obtained in a previous survey carried out in the northern Iberian peninsula by the same PCR-RFLP method (88%; Ruiz-González *et al.*, 2008).

Further analyses are needed to understand if these results depend on overlong interval between consecutive surveys - causing DNA degradation in the older faeces -, an overlong storage period of the samples before analysis - which could increase the probability of DNA degradation -, a high rate of faeces misidentification - as reported for low density pine marten populations in Great Britain (Davison *et al.*, 2002) -, or a combination of these factors.

In man-made habitats, the residual patches

of semi-natural vegetation provide cover, suitable den-sites and food to many species, particularly elusive carnivores. Until a few years ago, in the study area four medium-size carnivores had been reported, the red fox, the stone marten, the polecat and the badger (*Meles meles*). The latter mainly rely on earthworms and maize (Balestrieri *et al.*, 2004), whilst little is known about the polecat, the presence of which has not been confirmed by our analyses; the diet of the red fox and *Martes* species widely overlap, probably as a consequence of the low trophic diversity of the agricultural areas in the plain (authors' unpublished data). The two *Martes* species are considered to compete for the same resources (Delibes, 1983), even if they can co-exist in many areas (Genovesi, 1993; Ruiz-González *et al.*, 2008), exploiting the same microhabitats (Posluszny *et al.*, 2007). In the study area, according to their body weight ratio, the fox is likely to play the role of the dominant species (Prigioni *et al.*, 2008), whilst the stone marten should be the least competitive (Hermann, 1994). We argue that, in conditions of food shortage, the entry of the pine marten could alter the existing ecological relationship within the carnivore guild to the detriment of the stone marten.

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