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# Trophic habits of *Muscardinus avellanarius* (Mammalia Gliridae) as revealed by multiple stable isotope analysis

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Multiple stable isotope analysis was used to investigate the diet of the common dormouse Muscardinus avellanarius in a mixed Mediterranean forest.  $\delta^{13}C$  and  $\delta^{15}N$  values of dormouse tissues compared to those of the vegetal and animal sources available in the area showed that the isotopic composition of both adult and juvenile M. avellanarius could be explained by a mixture of vegetal and animal organic matter. The isotopic composition of the assimilated nitrogen was mostly explained by the organic matter from flora. Among the vegetal components, flowers of Fagus silvatica, Quercus robur, and the rarer Crataegus oxyacantha, berries of Ilex aguifolium and acorns of O, robur were found in the adult diet, while juveniles relied mainly on flowers of F. silvatica and acorns of Q. robur. Organic matter from eggs of passerines (Parus caeruleus and P. major) sharing nest-boxes affected the carbon isotopic signals in the assimilated fraction. The common dormouse in the mixed holly forests of the northern Sicily feeds primarily on the vegetal species which are abundant in its habitat. However, the diet of this small arboreal mammal cannot be fully characterised without considering the contribution of animal organic matter represented by bird eggs.

KEY WORDS: Muscardinus avellanarius, diet, stable isotopes, Mediterranean woodlands.

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### INTRODUCTION

The diet preferences of the common dormouse (*Muscardinus avellanarius*) are still poorly known, though it appears to rely primarily on vegetal organic matter (RICHARDS et al. 1984, BRIGHT & MORRIS 1993, VOGEL 1997). In addition, there is no information on its trophic preferences, particularly in Mediterranean areas, and there is no substantial data on the potential role of bird organic matter in the dormouse diet.

Experimental studies on cavity-nesters are often based on wooden nest-boxes to produce a desired species colonisation (Dhondt & Eyckermann 1980, Morris et al., 1990). The use of such artificial devices has become a widespread and practical way to model ecological interactions occurring in woodland ecosystems (Blondel 1995, Newton 1998). Recently, the competition for space among occupants of artificial nest-boxes has been reviewed by Juskaits (1995, 1999), who reported that common dormice occupying artificial nests affected the breeding success of some bird species. In addition, such unwanted interactions between dormice and birds competing for artificial nest-boxes (viz. "dormousitis") have been identified since the mid 1980s (Vaughan 2001). Other authors have expressed doubts about whether *M. avellanarius* can eat bird eggs and nestlings (e.g. Likhachev 1971 in Juskaitis 1995 or Vogel 1997). To date, the putative predation of *M. avellanarius* upon small forestry passerines has been predicted but never proved.

Insights into the trophic relationships and feeding habits of the dormouse can be gained by using ratios of naturally occurring stable isotopes, often of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N), in specific tissues (Deniro & Epstein 1978, 1981). This method is based on the correlation of stable isotope ratios in the tissues of animals with those in their diet (Minagawa & Wada 1984), and has allowed ecologists to examine complicated dietary frames of organisms living in multi-trophic source environments (Michener & Shell 1994).

Carbon and nitrogen isotopes have been successfully used to investigate diets in many species of terrestrial mammals (Des Marais et al. 1980, Fleming et al. 1993, Koch et al. 1995, Ambrose & Deniro 1996, Ben-David et al. 1997) and birds (Schirnding et al. 1982, Carter & Sealy 1986, Gould et al. 1997). For example, such techniques provided support for the assertion that rats rely on seabirds and that they can potentially affect seabird densities (Stapp 2002, Major et al. 2007). However, stable isotopes have never been used to investigate trophic preferences in small woodland mammals.

The main objective of the present paper is to study the feeding habits of *M. avellanarius* in a Mediterranean habitat. The specific aims were: (i) to search for the potential dietary resources of the common dormouse among those available on site, and (ii) to investigate whether the organic matter of birds (*Parus caeruleus* and *Parus major*) using the same artificial breeding space could represent a source of organic matter that could be exploited by the common dormouse. Ultimately, the study of the trophic niches of a forest species like the common dormouse may allow us to gain information that can be used for the conservation and management of biodiversity in Mediterranean woodlands.

### MATERIAL AND METHODS

Study area and sample collection

The study area was in the Madonie mountain range, a Natural Regional Park (40,000 ha in size) in northern Sicily (37°50'N; 14°05'E). The vegetation of the dolomite massifs in this range is arranged along several altitudinal belts (Pignatti 1997, Raimondo 1998), commencing with the high altitude (1300-2000 m a.s.l.) beech forests (Anthrisco-Fagetum aceretosum) of the Subatlantic belt, extending to the Colchic belt (1000-1350 m a.s.l.) and then to low altitude (50-800 m a.s.l.) mixed evergreen oak wood (Erico-Quercion ilicis) of the Mediterranean belt. Samplings were carried out in four 1-ha grid areas, where we positioned artificial nest boxes (Sarà et al. 2005). The study areas were characterised by holly forest (Ilici-Quercetum petraeae) in the Colchic belt, which is a localised pre-glacial association of evergreen plants (Ilex aquifolium, Daphne laureola, Taxus baccata, Buxus sempervirens, and Ruscus aculeatus) mixed with temperate deciduous oaks (Quercus petreae and Q. robur). A more detailed description of the study areas is reported in Sarà et al. (2001, 2005).

Field seasons of 2002 and 2003 were used to collect adults and juveniles of common dormice found dead inside the nest boxes and the muscular tissues of dead *Parus caeruleus* adults and eggs of both tit species from destroyed and/or abandoned nests built inside nest-boxes. In this preliminary contribute other putative animal food sources (i.e. insects) were not sampled due to the limited budget for isotopic analyses. Dominant vegetal sources of organic matter (leaves, flowers and acorns from plants) in the four nest-box grids were collected contextually, while the canopy coverage of the arboreal species in the  $20 \times 20$  m patches around every tree hosting a nest-box was also recorded.

# Stable isotope ratios and analysis

The rationale behind stable isotope analysis lies in the fact that most elements of biological importance have at least two stable isotopes. The ratio of these two stable isotopes can be modified during incorporation into living tissues due to metabolism (this modification is termed isotopic fractionation). The difference between the fractionation of any two stable isotopes (e.g. those of carbon or nitrogen) generates a ratio reflecting the relative abundance of the two stable isotopes (EHLERINGER et al. 1986). These differences are normally very small and they are expressed as the ratio of the heavier to the lighter isotope (Delta value; S) and denoted as the deviation per mil (‰) from an established standard (as depicted in Eq. 1 below). When the heavier isotope concentrates in the component of interest, then that component is commonly referred to as enriched. Contrarily, if the light isotope is the one that concentrates, then the component is referred to as depleted.

The implication of this chemical phenomenon is that each biological organism incorporates isotopes of a given element (e.g. carbon) at a specific ratio (signature), which approximates to the net or mean ratio of isotopes present in its diet (DENIRO & EPSTEIN 1978, 1981). The standard paradigm in isotopic dietary analysis is that metabolic fractionation follows a general scheme, producing an enrichment of the assimilated fraction for food; this fraction is light (1.5‰) for carbon and more pronounced (about 3.4‰) for nitrogen (Post 2002).

Muscle samples, aborted eggs from the two bird species, as well as leaves, flowers and acorns from all sampled plants used in isotopic analyses were sampled and immediately frozen (– 20 °C). The samples were rinsed with distilled water, acidified in HCl, re-rinsed in distilled water and dried at 60 °C until they reached a constant weight. They were then ground with a pestle and mortar. The isotopic analyses were performed by means of a Finnigan Delta-S isotope ratio mass spectrometer. Isotopic values were expressed in parts per mil as deviations from standards (Peedee belemnite limestone for  $\delta^{13}$ C and nitrogen in air for  $\delta^{15}$ N):  $\delta^{13}$ C or  $\delta^{15}$ N = [(Rsample/Rstandard) – 1] \* 103; where R =  $^{13}$ C/ $^{12}$ C or  $^{15}$ N/ $^{14}$ N (Eq. 1).

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Statistical analysis and data elaboration

The mixing model approach (PHILLIPS & GREGG 2003) was used in order to identify the organic sources most important to the isotopic composition of *M. avellanarius*'s assimilated fraction. Because there were more potential sources than stable isotopes analyzed, no single solution could be calculated from any one model. In the model, isotopic signals of adult and juvenile *M. avellanarius* were the targets, whereas isotopic signals of the pooled available vegetal matter, blue tits and coal tits were the main end-members. Models were run using the Iso-source routine developed by PHILLIPS & GREGG (2003). This method lists all possible combinations of sources (from 0 to 100%) for each contributor in increments of 1%. Those which produced the observed consumer's isotopic signature within a small tolerance range were considered to be feasible solutions. Lastly, these latter combinations are reported as the mean value plus the trimmed 1 and 99 percentile range (percentile; ABED-NAVANDI & DWORSCHAK 2005) of source contributions.

The amount of isotope fractionation between diet and common dormice tissue may have been different for the different tissues but there were no data to determine the presence or magnitude of such variation. For this reason, and because of the high variability in isotopic signatures of some potential prey items and the lack of other potential prey (e.g. insects), estimates of the relative contributions of different foods to a common dormouse diet using a multiple-source mixing model were not attempted (cf. Ben-David et al. 1997). Instead, this study sought to identify qualitatively the differences in isotopic signatures of a first sample of foods potentially entering into the diet of common dormouse in the studied habitat.

### RESULTS

Sources of organic matter in the Mediterranean holly forest

Seven vegetal species potentially contributing to the diet of M. avellanarius were recorded in the study area. Together, these species comprised 10 different forms of organic matter in the form of leaves, flowers, acorns and berries (Table 1). Carbon isotopic values of the vegetal sources ranged from depleted values measured in Pyrus sp. leaves (– 31.8%) to more enriched values of F. silvatica flowers (– 25.0%). Pyrus was also the most depleted source in terms of  $\delta^{15}N$  (– 1.7%), while acorns of F. silvatica represented, once again, the most enriched vegetal source, with an average 2.4%  $\delta^{15}N$ . The components of F0. F1. F2. F3. F3. The components of F3. F4. F5. F5. F6. F7. F7. F8. F8. F9. F9.

Isotopic composition of M. avellanarius and the contribution of organic matter sources to its diet

The isotopic content of *M. avellanarius* (Table 1) was depleted in adult specimens, while it was approximately 0.5% more enriched for both carbon and nitrogen in juveniles. Indeed, carbon isotope values were – 25.7% and – 25.1% respectively in adults and juveniles, while the mean values of nitrogen isotopes were 4.1% and 4.8%, respectively. Applying the accepted fractionation values of 1.5% for car-

Table 1.

Vegetal and animal sources sampled for the investigation of the isotopic content in the *M. avellanarius* diet.  $\delta^{13}$ C and  $\delta^{15}$ N = carbon and nitrogen isotopic values  $\pm$  SD, in the potential vegetal and animal foods (end-members in the mixing models) and in the consumer (target in the mixing models); Pool = mean value of the whole end-members and targets analysed in the present study; (\*) = three samples of 1 g.

Code	Source	Type	Collection	n	$\delta^{13}C$	$\delta^{15}N$
	End-members					
Ia-fl	Ilex aquifolium	flower	May	10	$-~28.9~\pm~0.2$	$-~1.5~\pm~0.3$
Ia-be	Ilex aquifolium	berry	Sep	15	$-30.4 \pm 0.4$	$1.3\pm0.4$
Co-fl	Crataegus oxyacantha	flower	May	10	$-30.7 \pm 0.8$	$0.8\pm0.3$
Fs-ac	Fagus silvatica	acorn	Sep	7	$-26.5 \pm 0.1$	$2.4~\pm~0.2$
Fs-fl	Fagus silvatica	flower	May	6	$-25.0 \pm 0.3$	$0.6~\pm~0.3$
Mal-frt	Malus sp.	fruit	Sep	2	$-29.4 \pm 0.7$	$0.4~\pm~0.4$
Py-fl	Pyrus sp.	flower	May	10	$-31.8 \pm 0.5$	$-~1.7~\pm~0.3$
Rc-be	Rosa canina	berry	Sep	12	$-~28.5~\pm~0.4$	$2.2~\pm~0.1$
Qr-fl	Quercus robur	flower	May	10	$-29.7 \pm 0.6$	$0.7\pm0.5$
Qr-ac	Quercus robur	acorn	Sep	8	$-26.3 \pm 0.5$	$0.7~\pm~0.2$
	Pool				$-28.7 \pm 2.1$	$0.6 \pm 1.4$
	End-members					
Pc-ad	Parus caeruleus	adult	May, June	4	$-24.8 \pm 1.4$	$5.0 \pm 2.2$
Pc-egg	Parus caeruleus	egg	May, June	4	$-25.3 \pm 0.9$	$5.0 \pm 1.1$
Pm-egg	Parus major	egg	May	4	$-28.3 \pm 1.7$	$5.0 \pm 0.7$
	Pool				$-26.4 \pm 2.2$	$5.0 \pm 1.4$
	Target					
Ma-ad	Muscardinus avellanarius	adults	Mar, May, Oct, Dec	4	- 25.7 ± 0.9	$4.3 \pm 0.5$
Ma-juv	Muscardinus avellanarius	juveniles	Feb, Mar, Apr, Oct	4	- 25.1 ± 1.0	$4.8\pm0.7$
Ma-fae	Muscardinus avellanarius	faeces	June	3(*)	$-28.9 \pm 1.2$	$-0.3 \pm 0.9$
	Pool				$-25.9 \pm 1.5$	$2.9\pm2.7$

bon and 3.4% for nitrogen (Post 2002), the mixing model analysis predicted that the diet of M. avellanarius adults consisted mainly of vegetal organic matter (Table 2), although organic matter from bird eggs could possibly have contributed to the diet as well. In particular, both juvenile and adult M. avellanarius appeared to derive most of their assimilated carbon from animal organic matter (> 60% in adults and ca 80% in juveniles). In contrast, vegetal organic matter contributed largely to the nitrogen fraction (> 80%) of the diet of both life-stages.

Table 2.

Contribution (mean %) and trimmed percentiles (percentile 1% and percentile 99%) of organic sources available in the study areas and potentially entering the diet of adults and juveniles of *M. avellanarius*, as calculated by the mixing models (All-veg = all vegetable sources pooled together; Pceggs = *Parus caeruleus* eggs; Pm-eggs = *Parus major* eggs).

		Adults Carbon		Juveniles Carbon					
Source	mean %	percentile 1%	percentile 99%	mean %	percentile 1%	percentile 99%			
All-veg	34.3	0.0	69.0	19.9	0.0	40.0			
Pc-eggs	25.8	19.0	32.0	56.7	52.0	61.0			
Pm-eggs	39.9	0.0	80.0	23.4	0.0	47.0			
		Adults Nitroger	ı	Juveniles Nitrogen					
Source	mean %	percentile 1%	percentile 99%	mean %	percentile 1%	percentile 99%			
All-veg	93.5	93.0	94.0	81.5	81.0	82.0			
Pc-eggs	3.3	0.0	7.0	9.3	0.0	19.0			
Pm-eggs	3.3	0.0	7.0	9.3	0.0	19.0			

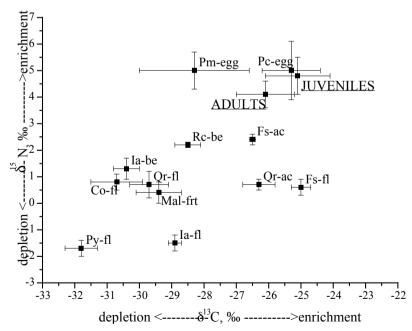


Fig. 1. — Biplot  $(\delta^{13}\text{C-}\delta^{15}\text{N})$  of the isotopic compositions of the most abundant organic sources potentially available to *M. avellanarius* adults and juveniles. The codes of animal and vegetal sources are as reported in Table 1.

Among the vegetal items, flowers of *F. silvatica, C. oxyacantha, Q. robur*, berries of *I. aquifolium* and acorns of *Q. robur* appeared to make up the better part of the diet of adult dormice. The remaining available vegetal end-members did not appear to be sources in the *M. avellanarius* diet, or it is likely that they represented only occasional foods (Fig. 1). The contribution of *P. major* eggs to the diet was slightly higher than that of *P. caeruleus* eggs in adults (Table 2). Thus, although *P. major* eggs were less common in the area — the density in the nest-boxes from 1996 to 2003 was  $0.53 \pm 0.51$  (range: 0-1) breeding pairs compared to  $9.65 \pm 3.72$  (range: 4-18) *P. caeruleus* pairs — this organic matter appeared to be a more efficient source in the diet of the adult dormouse.

With regards to young dormice, the individuals analysed in the present study were weaning pups still dependent on the mother. The mother transferred organic matter to juveniles by means of maternal milk, and the resulting isotopic composition was mainly explained, with regards to carbon, by egg organic matter (80.1%). Contrary to the isotopic frame predicted by the mixing models for the adults, *P. caeruleus* eggs represented the dominant organic source in the assimilated carbon fraction of juvenile dormice (56.7%), whereas a lesser amount of carbon (23.4%) was from *P. major* (Table 2). Accordingly, the nitrogen fraction in juveniles was largely explained by vegetal organic matter (81.5%), in which flowers of *Fagus silvatica* and *Quercus robur* acorns represented the better part of this assimilated fraction.

# DISCUSSION

These isotopic results support former analyses of the foraging behaviour of dormice in England (BRIGHT & MORRIS 1993). Dormice were highly selective of soft mast and used trees in a significantly different proportion to their percentage cover, thus showing an opportunistic behaviour (sensu Rosenweig 1981). In the case of English woodlands, both rare tree species such as *I. aquifolium*, and common tree species such as *Q. robur*, were preferentially utilized depending on the season. As suggested by the isotope analysis, the common dormouse also exploits dominant plant species in its habitat in the Mediterranean mixed holly forest, together with some rare species. Accordingly, in our study areas, the canopy coverage around the 104 nest-boxes sampled was mainly composed of *Q. robur* (37.9%), *I. aquifolium* (37.7%) and *F. silvatica* (16.1%), and only *C. oxyacantha* was quite rare (2.4%) among the vegetal sources, in the diet. Stable isotopes confirmed therefore the use of oak acorns, as suggested by BRIGHT & MORRIS (1993).

Exploitative competition between two small cavity-dwellers (*Parus caeruleus* and *M. avellanarius*) for breeding space, represented by wooden nest-boxes, has been highlighted in Mediterranean woodlands (SARA et al. 2005). In this particular study, a high percentage of nest failure was observed due to destruction and predation by *Martes martes* (15%) and unknown arboreal rodents (23%). *M. avellanarius* was suggested as the cause of this high percentage of nest failure, a hypothesis which presumes that exploitation competition for nest-boxes also extends to a predatory interaction. Because we were interested to such a particular aspect, we sampled no other animal sources that might potentially enter the diet of the common dormouse. However, the stable isotope approach gave some insight into the debated role of animal organic matter represented by some small passerines sharing the same habitat and breeding site.

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According to isotope analysis, in the Mediterranean mixed holly forest common dormice prey on the eggs of passerines nesting in nest-boxes, thus confirming former studies (JUSKAITIS 1995, SARA et al. 2005) suggesting a high percentage of nest failures due to this small arboreal rodent. Although it is not possible to generalize our result to other areas, it is becoming apparent that nest-boxes set to enhance the density of some species or, additionally, to study their life history in the field, may lead to unwanted effects. Paradoxically, such conflict is more difficult to solve when interacting species are both protected or rare as in the present case or in that cited by Vaughan (2001).

In conclusion, the common dormouse in the mixed holly forests of northern Sicily feeds primarily on the vegetal species which are abundant in its habitat. However, the diet of this small arboreal mammal cannot be fully characterised without considering the contribution of animal organic matter. In this case, the animal matter, represented by tits eggs, seems to be an important source during spring, when with respect to plants, it is the most significant food transferred to lactating juveniles. Exploitation of seasonal food sources that are available in the mixed holly forest (i.e. flowers, leaves and eggs in spring; berries and acorns in autumn), is likely to broaden the time-span of the reproductive season all year-round (SARA 2000), and to maintain an equivalent level of reproductive success across seasons (3.79  $\pm$  0.97 youngsters; n = 14 litters in April-June, vs 4.20  $\pm$  1.08 youngsters; n = 15 litters in October-December).

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