

Different habitats, different pressures? Analysis of escape behaviour and ectoparasite load in *Podarcis sicula* (Lacertidae) populations in different agricultural habitats

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Abstract. Human agricultural activities can deeply alter the environment thus provoking major impacts on a variety of organisms. Agricultural habitats however can be very different from one another in terms of habitat structure and management intensity, presenting varying pressures and/or benefits for different species. Agro-ecosystems can have opposing effects on reptiles and in some circumstances the presence of a species can even be enhanced by agricultural practices. We focused our study on *Podarcis sicula*, a relatively widespread lacertid lizard commonly present in agro-environments in Italy. We examined escape behaviour, caudal autotomy rates and ectoparasite load (tick infestation) in populations living in two different land uses, olive tree plantations and vineyards. All three aspects seemed to be deeply influenced by habitat structure. Predation pressure, as evaluated by tail break frequency, was lower in olive tree plantations, the most structurally complex habitats. In this type of habitat lizard escape behaviour was characterised by a clear preference for olive trees as refuges: individuals ran farther distances on average to reach the trees and hid inside them for a relatively long time. In vineyards, on the contrary, a less clear escape strategy was observed, showing a use of more temporary refuges. Also tick (*Ixodes ricinus*) infestation differed among land uses, being higher in olive tree plantations, probably in relation to vegetation cover features. Differences were found also between managements (with a higher tick load in traditional cultivations) and sexes, with males being more parasitized.

Keywords: agro-environment, antipredatory behaviour, ectoparasite, lizard, parasite infection.

Introduction

Human activities deeply alter the environment creating novel habitats, modifying the balance among the pre-existing ones, and thus having important ramifications for a diversity of taxa. Agriculture, in particular, can cause considerable and very quick environmental alterations (Elliott and Cole, 1989); this is especially true for intensive agriculture characterised by both huge extensions of exploited lands and large use of chemicals and machinery. The impact of anthropogenic disturbance and environmental alterations upon reptile communities and species can vary (Smart, Whiting and Twine, 2005). Agricultural activities can have opposing effects on reptiles causing the decrease in species diver-

sity in some circumstances (Glor et al., 2001) but enhancing the presence of other species (Hódar, Pleguezuelos and Poveda, 2000). However, even considering a ubiquitous species able to live in most of the agro-environments, it is reasonable to hypothesize a tuning of its responses to the pressures varying with different land uses and managements.

Podarcis sicula, is the most abundant lacertid lizard in Italy along with *P. muralis* (Avery, 1978) and is a quite common species in anthropic environments. Due to its biological characteristics such as high thermophily, it is particularly well adapted to living in open habitats. In central Italy, where it is usually present at low altitudes (Corti and Lo Cascio, 2002), it is the most abundant lizard in agricultural habitats. In spite of the numerous reports of its ubiquitous presence, very little is known about the ecology of the Italian Wall Lizard in agro-environments. All these elements make *P. sicula* an interesting model species to investigate ecological aspects in agricultural habitats.

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For organisms like lizards that are in an intermediate position in the trophic chain, predation pressure is a key ecological aspect. In the study region, for instance, *Podarcis* species are preyed upon by a lot of vertebrates such as snakes and a variety of birds. In particular, in agricultural ecosystems, lizards are key links among trophic levels over the long term, and changes in their population densities can have cascading effects on other trophic levels (Martín and López, 1996a; Díaz, Monasterio and Salvador, 2006). Indeed, the ectothermy and the largely insectivorous diet of these vertebrates allow them to attain high densities in low productivity ecosystems because they can successfully exploit a large prey base (small arthropods) that most endothermic predators cannot energetically afford to feed on (Regal, 1983). These high densities, in turn, provide a resource for predators. Escape behaviour is a complex adaptive process (Delibes and Blázquez, 1998) that can be influenced by habitat selection (Bulova, 1994; Martín and López, 1995; Downes and Shine, 1998). Escape strategies depend on the assessment of predation risk and on the evaluation of costs and benefits deriving from the different possible tactics, such as running towards a refuge or adopting a cryptic behaviour. Escaping and refuging, in fact, can have high costs in terms of both energy spent and time reduced from other important activities (Lima and Dill, 1990; Cooper, 1999; Martín and López, 2000) such as thermoregulation, feeding and mating.

Another pressure that could be linked to a specific habitat is parasite infection. In fact, the presence and also the impact of parasites may be spatially dependent (Bouma et al., 2007). In particular, ectoparasites like ticks are associated with animal host availability as well as with a number of environmental factors (Lubelczyk et al., 2004; Medlock et al., 2008). Cover thickness and mat depth of vegetation and also soil drainage and vegetation type, shaping microclimatic and humidity conditions, can affect the presence and consistence of tick populations (Medlock et al., 2008). All these factors in agro-

environments are strictly dependent on the type of cultivation and management. Even if host-parasite interactions are in general very complex and sometimes not easy to highlight (Bull and Burzacott, 1993), they play an important role in lizard ecology. In fact, parasite infections in lizards can result in both physical damage (Shall, Bennett and Putman, 1982; Bull, Burzacott and Sharrad, 1989; Sorci and Clobert, 1995; Oppliger, Celerier and Clobert, 1996) and, subsequently, in social costs such as smaller home-ranges (Main and Bull, 2000) and reduced social status (Schall and Houle, 1992; Dunlap and Schall, 1995).

In this paper we examined both predation pressure and ectoparasite load (tick infestation) in *P. sicula* populations living in two different agricultural habitats. Predation pressure was evaluated by tail break frequency while lizard antipredatory response was assessed by the observation of escape behaviour. Pressures were analysed in relation to land use and management intensity.

Materials and methods

Study sites

The study was performed in two agricultural areas in Tuscany (Pistoia), Central Italy. Two typical land uses of the Tuscan agricultural landscape were analysed: olive tree plantations (O, *Olea europaea*) and vineyards (V, *Vitis vinifera*). For each of these two different kinds of management were considered: the "intensive" one (Int) in which chemicals and machinery are used and the "traditional" one (Trad), characterised by no chemicals and machineries and by the maintenance of grass soil cover. In Casalbosco factory (43°56'12.22"N, 10°59'59"; 150 ha), the main study area, five sites were surveyed: two intensively managed vineyards (Int-V), two intensively managed and one traditionally managed olive tree plantations (Int-O and Trad-O, respectively). In the second study area, at about 4 km from Casalbosco, a sixth site (43°57'06"N, 10°56'50"E; 2.4 ha), a traditional vineyard (Trad-V), was surveyed.

Study species

Podarcis sicula is a medium sized lacertid lizard (in the present study, males: $n = 62$, mean SVL \pm SD = 67.38 \pm 4.34 mm; females: $n = 74$, mean SVL \pm SD = 61.41 \pm 4.39 mm) mainly distributed in continental Italy, in most of the surrounding islands and in several localities along the

eastern coast of the Adriatic sea. In Tuscany, where it is referred to the subspecies *P. s. campestris* (De Betta, 1857), it is largely widespread in habitats with high levels of human activity.

Sampling methods

Lizards were collected in 2007 from early Spring to Summer. Each lizard was measured (SVL), the sex, the amount of ticks and the presence of broken/regenerated or entire tail were recorded. Ticks were collected and then identified at species level following Manilla (1998). After being processed, lizards were released at the point of capture. We used tail break frequency as an indicator of predation pressure (Turner et al., 1982; Pérez-Mellado, Corti and Lo Cascio, 1997; Diego-Rasilla, 2003).

Escape behaviour was analysed in 6 consecutive days in late August 2008, at the end of lizard mating season. In mid August-September juvenile lizards are very active and easily detectable. In the present study we focused our attention on this age class for which predation pressure is particularly high (Martín and López, 1996b). Moreover, by considering only juveniles with entire tails, we both excluded the influence of tail condition on the escape response (Martín and Salvador, 1997; Martín and Avery 1998) and tried to minimize possible influences of previous predation experiences. In order to avoid confounding effects in data gaining, sampling was performed by the same surveyor (M.B.), behaving and dressing in the same way. After being sighted with binoculars (at approximately 4 m), each lizard was approached by walking towards it at a constant speed, as to simulate a predatory attack (Martín and López, 1999a). We recorded the flight distance (the distance the lizard allowed the observer to get near before starting running) and the total distance fled by the individual before entering a refuge or stopping running (Bulova, 1994; Cooper, 1997; Diego-Rasilla, 2003; Carrettero et al., 2006). If the observed lizard hid, we also recorded the time of appearance (snout visible) from its shelter (Martín and López, 1999b; Carrettero et al., 2006). After each escape sequence the temperature of air (T_a , 1 m above the ground) and of the substrate (T_s) were recorded using a digital thermometer. In order to minimize the risk of repetitive sampling of the same individual we surveyed each site only once and within the same site we walked linear paths that were at least 10 m apart, so we considered data as independent.

Statistical analyses

In order to analyse the tail break occurrence in relation to SVL, sex, land use and management we performed a LOGIT Generalized Linear Model (GLZ): we used tail break occurrence as binary dependent variable, SVL as continuous predictor and sex, land use and management as categorical factors.

General Linear Models (GLM) were run to search for possible differences in SVL, in relation to land use and management (fixed factors): the two sexes were analysed separately. To normalize data, SVL values were log-transformed (Kolmogorov-Smirnov: females, $n = 74$, $d = 0.094$, $P =$

0.465; males, $n = 62$, $d = 0.061$, $P = 0.975$). Tukey HSD tests were used for post-hoc comparisons.

Pearson correlations and GLMs were performed in order to analyse lizard escape behaviour. Even if multiple comparisons were run, all the P values of Pearson correlations resulted non-significant (see Results) and thus it was not necessary to apply any multiple comparison correction. Flight distance, distance fled and appearance time were analysed in relation to T_a , T_s , refuge/stop tactic and land use. G test was used to compare the frequencies of the different escape behaviours between land uses.

Owing to the high number of lizards on which no ticks or just one tick was recorded, parasite load was not normally distributed even after log-transformation (Kolmogorov-Smirnov: $n = 136$, $d = 0.200$, $P < 0.01$). For this reason we performed a GLZ assuming a Poisson distribution of the data. We considered the number of ticks recorded on each individual in the 6 different sites as dependent variable, logSVL as a continuous predictor, and management, land use and sex as categorical factors. To avoid possible confounding effects due to the differences in SVL existing between males and females, the same analysis was repeated for the two sexes separately.

T -tests were used for comparisons between two groups. Statistica 7.0 package was used for all the analyses except for G test, performed by Pop-Tools.

Results

GLM on SVL values revealed differing patterns occurring for each sex. In fact, males showed significantly different sizes between cultivations ($n_{O-Males} = 45$, $n_{V-Males} = 17$, $F = 7.8$, $P = 0.007$) but not between managements ($n_{Trad-Males} = 23$, $n_{Int-Males} = 39$, $F = 0.1$, $P = 0.963$) (table 1, data not log-transformed are reported). On the contrary, females did not differ between cultivations ($n_{O-Females} = 38$, $n_{V-Females} = 36$, $F = 3.1$, $P = 0.083$) even if they were slightly bigger in vineyards, but SVL values were significantly higher in intensively managed sites rather than in the traditionally managed ones ($n_{Trad-Females} = 24$, $n_{Int-Females} = 50$, $F = 6.7$, $P = 0.012$) (table 1).

LOGIT GLZ performed on broken/regenerated tail occurrence revealed that lizards collected in the vineyards had more frequently regenerated tail ($n_O = 83$, $n_V = 53$, Wald = 4.519, $P = 0.034$). On the contrary, logSVL ($n = 136$, Wald = 0.493, $P = 0.483$), management ($n_{Trad} = 47$, $n_{Int} = 89$, Wald = 0.058,

Table 1. SVL of lizards in olive tree plantations (O), in vineyards (V), in traditionally managed sites (Trad) and in intensively managed sites (Int). Sample size and SVL mean values ± SD are reported for each category.

SVL (mm)	O		V		Trad		Int	
	<i>n</i>	Mean ± SD						
Males	45	66.55 ± 3.67	17	69.56 ± 5.27	23	67.09 ± 4.71	39	67.54 ± 4.17
Females	38	60.43 ± 3.75	36	62.45 ± 4.83	24	59.32 ± 2.67	50	62.42 ± 4.72

Table 2. GLM performed on flight distance, distance fled and appearance time in relation to air and soil temperature, land use and refuge/stop tactic (*n* = 115; *n*_O = 74, *n*_V = 41, *n*_{Refuge} = 89, *n*_{Stop} = 26) (O = Olive tree plantations; V = Vineyards).

	Flight distance		Distance fled		Appearance time	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Intercept	1.342	0.250	0.036	0.850	0.958	0.331
<i>T</i> _a	0.364	0.548	2.385	0.127	1.142	0.288
<i>T</i> _s	0.196	0.659	1.325	0.253	1.840	0.179
V/O	0.122	0.727	0.113	0.738	8.162	0.005
Flight dist.	–	–	0.002	0.967	0.206	0.651
Dist. fled	0.002	0.967	–	–	0.196	0.659
Appearance time	0.206	0.651	0.196	0.659	–	–
Refuge/stop	0.797	0.375	0.617	0.434	<0.001	0.982
V/O*refuge/stop	1.678	0.199	4.854	0.030	0.028	0.868

P = 0.810) and sex (*n*_{Males} = 62, *n*_{Females} = 74, Wald = 0.710, *P* = 0.400) did not show any significant effect.

No significant correlations were found among flight distance, distance fled, *T*_a, *T*_s and appearance time (*P* > 0.05 in all cases): only a predictable correlation between *T*_a and *T*_s was found (*n* = 115, Pearson *r* = 0.896, *P* < 0.001; mean values ± SD: *T*_a = 29.82 ± 3.15°C, *T*_s = 27.53 ± 3.48°C). GLM performed on flight distance (mean values ± SD: *n*_O = 74, 181.028 ± 76.857 cm; *n*_V = 41, 178.367 ± 81.868 cm) did not give significant results (table 2). The same analysis performed on distances fled by lizards (mean values ± SD: *n*_O = 74, 223.102 ± 103.919 cm; *n*_V = 41, 182.972 ± 72.699 cm) revealed a significant interaction between the land use and the choice of escaping into a refuge or stopping without doing so (table 2 and fig. 1). In olive tree plantations lizards fled, on average, a longer distance to reach a refuge while a shorter one when deciding just to stop (mean values ± SD: *n*_{O-Refuge} = 62, 230.952 ± 136.319 cm; *n*_{O-Stop} = 12, 176.000 ± 61.537 cm; *t* = -0.829, *P* = 0.411); an opposite pattern characterised vine-

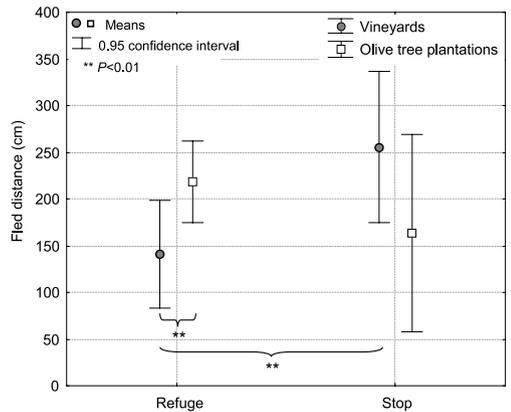


Figure 1. Significant interaction between the distance fled by lizards and the choice of refuge/stop tactic in the two land uses, as revealed by GLM.

yards where individuals that stopped without hiding ran a longer distance than the ones that entered a refuge (mean values ± SD: *n*_{V-Refuge} = 27, 144.703 ± 84.856 cm; *n*_{V-Stop} = 14, 259.500 ± 100.955 cm; *t* = 2.854, *P* = 0.007) (fig. 1). Considering only lizards that hid, a *t*-test revealed that the distance run to reach a shelter was significantly longer in olive tree plantations than in vineyards (*n*_{O-Refuge} = 62, *n*_{V-Refuge} = 27, *t* = -2.227, *P* = 0.029). De-

spite this result, G test showed that the portion of lizards that escaped into a refuge was higher in olive tree plantations than in vineyards (olive tree plantation: $n_{\text{Refuge}} = 62$, $n_{\text{Stop}} = 12$; vineyards: $n_{\text{Refuge}} = 27$, $n_{\text{Stop}} = 14$; $G_{(\text{adj})} = 4.587$, $P = 0.032$). GLM analysis showed that appearance time (mean values \pm SD: $n_{\text{O}} = 74$, 128 ± 56 s; $n_{\text{V}} = 41$, 67 ± 23 s) was significantly higher in olive tree plantations rather than in vineyards (table 2).

Ticks we collected belonged to the species *Ixodes ricinus* L. (Acari: Ixodidae). GLZ performed on the number of ticks per individual showed significant differences in ectoparasite load between land uses (O > V), man-

agements (Trad > Int) and sexes (Males > Females); an influence of SVL was identified, too (table 3). The same analysis performed for the two sexes separately confirmed the significant difference among land uses for both males and females (table 3 and fig. 2), while it revealed an influence of SVL only for males and a significant difference in tick load between managements just for females (table 3); considering only females living in olive tree plantations, *t*-test revealed that Trad-O lizards were more parasitized than Int-O lizards (mean values \pm SD: $n_{\text{O-Trad}} = 15$, 3.267 ± 3.240 ; $n_{\text{O-Int}} = 23$, 0.522 ± 0.730 , $t = -3.940$, $P < 0.001$). Finally, focusing just on lizards

Table 3. Mean values \pm SD of the number of ticks per lizard and GLZ performed on the individual tick load in relation to LogSVL, land use, and management. The same analysis was also performed for males and females separately (O = Olive tree plantations; V = Vineyards; Trad = Traditional management; Int = Intensive management).

Tick load	All lizards		Males		Females	
	<i>n</i>	Mean \pm SD	<i>n</i>	Mean \pm SD	<i>n</i>	Mean \pm SD
O	83	3.70 \pm 5.91	45	5.47 \pm 7.28	38	1.61 \pm 2.48
V	53	0.08 \pm 0.27	17	0.18 \pm 0.39	36	0.03 \pm 0.17
Trad	47	2.79 \pm 4.34	23	3.57 \pm 5.37	24	2.04 \pm 2.99
Int	89	2.02 \pm 5.23	39	4.28 \pm 7.33	50	0.26 \pm 0.56
GLZ	Wald	<i>P</i>	Wald	<i>P</i>	Wald	<i>P</i>
Log SVL	25.377	<0.001	33.958	<0.001	0.550	0.458
O/V	99.713	<0.001	39.970	<0.001	106.276	<0.001
Trad/Int	22.393	<0.001	0.183	0.670	334.648	<0.001
Sex	47.072	<0.001	–	–	–	–

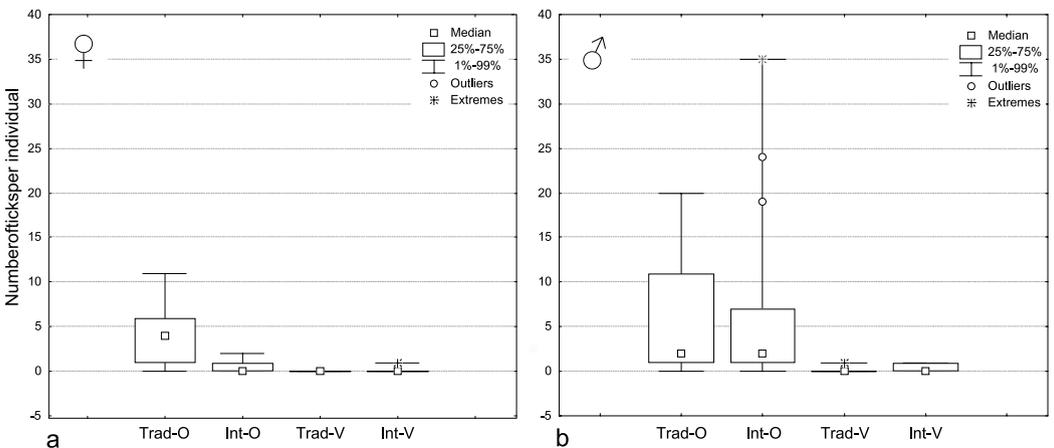


Figure 2. Tick load in females (a) and males (b) in the different kinds of surveyed land uses (O = Olive tree plantation, V = Vineyard) and managements (Trad = Traditional, Int = Intensive).

from olive tree plantations (in vineyards lizards were almost not parasitized, table 3), *t*-tests revealed for both sexes that individuals with broken/regenerated tail had not a significantly different tick load than individuals with entire tail (O-Females mean values \pm SD: $n_{\text{entire tail}} = 27$, 1.741 ± 2.740 , $n_{\text{broken tail}} = 11$, 1.222 ± 1.922 , $t = 0.524$, $P = 0.604$; O-Males: $n_{\text{entire tail}} = 25$, 4.960 ± 7.791 , $n_{\text{broken tail}} = 20$, 6.100 ± 6.735 , $t = -0.517$, $P = 0.607$).

Discussion

In vineyards the occurrence of broken or regenerated tails was significantly higher than in olive tree plantations. Even if other factors such as age of the animals or social structure can influence caudal autotomy rates, our result could also be interpreted as an indication of a higher predation pressure in the first form of land use. This, in turn, could be explained by a more consistent presence of predators and/or higher lizard detectability or a different quality of available refuges. The presence of predators, however, appeared to be greater in olive tree plantations. These sites in fact (and above all Trad-O) were characterised by the highest frequency of sightings of snakes such as *Hierophis viridiflavus* and *Natrix natrix*, both lizard predators, while in intensive vineyards *P. sicula* was the only reptile we observed; however, it is reasonable to suppose that birds feeding on *P. sicula* were the same in both vineyards and olive tree plantations because all the sampling sites inside Casalbosco farm were close to each other. On the other hand, an influence of habitat structure was also found in the analysis of lizard antipredatory response. In fact, in the two agricultural land uses we documented quite different escape behaviour of juvenile lizards. In olive tree plantations juveniles reacted to the simulated predatory attacks by running towards a definite refuge, the olive tree, and hiding inside it for a relatively long time. As a matter of facts, in these land uses the majority of juvenile lizards preferred to reach a refuge rather than

stopping without hiding, even if this meant, on average, traversing a greater distance. Most of these individuals (approximately 60%) choose olive trees (i.e., holes in the trunks or bushes at the basis of the tree) as refuges and, in almost 70% of the cases in which an alternative refuge was chosen, the nearest olive tree was farther away than 3 m in the direction of the escape trajectory, which is more than the average fled distance. On the contrary, in vineyards juveniles did not show such a clear strategy and the proportion of lizards entering a refuge or stopping was not significantly different. The former usually ran a shorter distance, hid for a short time and then started running again. In most of the cases individuals entered soil crevices or small grass patches, which indeed constitute temporary shelters rather than safe refuges. The interpretation of our results can likely be related to the constraints of the two different land uses. Vineyards are open habitats, characterised by relatively thin and linear vegetated rows intercalated with bare strips where soil is usually not covered by grass. Lizards are more easily detectable and long runs could expose them to a higher predation risk. In olive tree plantations the structural complexity made up of tree crown cover, grass soil cover (when present) and scattered bushes probably makes it advantageous for lizards to invest energy in fleeing a longer distance in order to reach a safe refuge. The hiding and "misleading" effect of the vegetation cover could be particularly effective against bird predators. Other studies had suggested that differences in vegetation cover may affect lizard escape behaviour, underlining that in more protected habitats lizards tend to be less wary (Snell et al., 1988; Bulova, 1994; Martín and López, 1995).

However, structural complexity of vegetation could also bring some disadvantages such as the higher presence of ectoparasites: in our study *Ixodes ricinus* infestation was almost nil for lizards in vineyards and significantly higher in olive tree plantations. It is known that the presence of ticks is related to vegetation and

in particular to grass, bushes and also to certain tree species. In particular a preference of the Ixodidae tick *I. rubicundus* was highlighted for wild olive trees (*Olea europaea africana*) (Fourie, Kok and van Zyl, 1991) while *I. pacificus* showed a preference for moss-covered oak trees (*Quercus* spp.) due to refugia existing in the bark and, intriguingly, to the presence of the Western Fence lizard (*Sceloporus occidentalis*) hosts on the lower-most surface of trees (Slowik and Lane, 2001). The other key factor affecting the presence of ticks, host availability (Medlock et al., 2008), varied between agricultural land uses too. The majority of *I. ricinus* nymphae and adult females parasitize relatively large mammals such as hares (*Lepus* spp.) and roe deer (*Capreolus capreolus*), their primary definitive hosts (Mejlon and Jaenson, 1997; Manilla, 1998). These mammals were observed much more frequently in olive tree plantations and especially in Trad-O where they probably dwell; on the contrary it is very likely that roe deer go inside vineyards just for feeding. We also found that tick infestation in *P. sicula* was significantly higher in males rather than in females. A similar trend was found in other lizard species in which differential levels of susceptibility to infection were related to complex interactions among effects of testosterone, male higher activity rates and control of larger home ranges which, in turn, may cause a higher exposure to parasites as well as a lower resistance to infection (Bauwens, Strijbosch and Stumpel, 1983; Salvador, Martín and López, 1995; Salvador et al., 1996; Olsson et al., 2000; Bouma et al., 2007). It is also probable that in *P. sicula* analogous mechanisms occur, especially during the mating season. Besides, as already observed in other lizards (i.e., Bull and Burzacott, 1993), we found a relation between body size and tick load in males. Bigger males usually have bigger home ranges, which could imply greater mobility and higher exposure to infestation. At the same time bigger individuals are probably more likely to maintain higher parasite loads (Bull and Burzacott, 1993; Amo, López and

Martín, 2005). On the other hand females, moving less than males, are perhaps more sensitive to the fine differences among managements. Indeed, we found that females had a significantly higher individual tick load in traditionally managed olive tree plantations rather than in intensive ones. Besides a likely larger presence of mammal hosts, in Trad-O no chemicals are used, more vegetation cover in terms of grass and bushes is present and the lower maintenance of the trees results in both a dense crown cover as well as in a mat of decaying leaf litter, all elements that are favourable to the presence of ticks (Fourie, Kok and van Zyl, 1991).

Our results suggest that habitat structure can deeply influence different aspects of lizard ecology. Therefore, complex environments like olive tree plantations can lower the risk of predation, as we observed, and are likely to offer better food resources and home ranges (Graziani et al., 2006) in comparison to vineyards; at the same time, however, vegetation complexity may also enhance the presence of parasites and competitors. Of course, further studies are needed to understand the trade off existing among benefits and costs deriving from living in a certain habitat. Additional observations, for instance, could help to clarify the SVL patterns recorded among different agricultural habitats. Some of the above mentioned ecological constraints (i.e., resource quality and availability, predation pressure, parasitism) or, more likely, an interaction among them could probably explain why lizards of both sexes tended to be bigger in vineyards rather than in olive tree plantations and also why females were significantly smaller in traditionally managed sites.

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Abetone, con particolare riferimento alla Legge Regionale 56/2000". All procedures conformed to Italian laws; samplings were authorized by Ministero dell' Ambiente e della Tutela del Territorio e del Mare.

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