

# Spatial and temporal segregation allows coexistence in a hybrid zone among two Mediterranean vipers (*Vipera aspis* and *V. latastei*)

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**Abstract.** Mediterranean vipers are ecologically very similar. When in contact, they constitute an exceptional model to study the effects of competitive interactions and niche segregation mechanisms. In High Ebro, distinct methodologies (random visual encounter, road sampling and radio-tracking surveys, captive maintenance, standardized transects and ad hoc field observations) were used to compare ecological traits (diet, micro-habitat, activity, reproductive and demographic) of *V. aspis*, *V. latastei* and hybrids among both species and determine competitive advantages of each form. Diet preferences were similar but few differences were found in feeding frequency and annual variation in diet consumption. Males of the three forms compete for areas with similar characteristics but differences were found mainly during summer. Significant differences in average movement rates and home range size were found among males. Fecundity and new-borns fitness were lower in *V. aspis* than in *V. latastei*, whereas hybrids had intermediate values. Male and female adult hybrids were more abundant than parental forms, whereas female and juvenile *V. latastei* and juvenile hybrids had high road-mortality. The three forms seem to be competing intensely for resources but the segregation on the spatial and temporal axes of their niches could be reducing interspecific competition and allowing coexistence. The use of different resources probably confers reproductive and demographic advantages for *V. latastei* and hybrids, respectively. Moreover, reproductive fitness of hybrids suggests the occurrence of endogenous selection. Comparative thermal and genetic studies are needed to clarify the limiting factors of vipers and to establish a model of the hybrid zone.

**Keywords:** activity, demography, diet, endogenous selection, fitness, micro-habitat, reproduction, *Vipera*.

## Introduction

Among the different forms of species interactions, such as predation, parasitism, or detritivory, interspecific competition and the mechanisms allowing coexistence between similar species have interested ecologists for a long time (Schoener, 1974). Interspecific competition may occur when two or more species with similar ecological requirements share the same area. If the resources are in short supply, one species may have lower fecundity, survival, or

growth as a result of resources exploitation or interference caused by the other species (Begon, Harper and Townsend, 1999). Even when resources are not in short supply, one species may be outcompeted by the other according to the competitive exclusion principle (Gause, 1935). However, natural selection may favour ecological divergence among species in coexistence to minimize the intensity of interspecific competitive interactions. Therefore, species tend to differ in their ecological niches (Ricklefs, 1998). Differences in resource use could have repercussions on reproductive and demographic traits of species in competition (e.g., Clarke, 1992). These traits might confer competitive advantages for one of the species in detriment of the others, for they affect life history traits and, thus, the dynamics of populations (Clutton-Brock, 1990).

Only a few studies have addressed the effects of interspecific competition on reproductive and demographic traits (e.g., Cameron et al., 2007; Gröning and Hochkirch, 2008). How-

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1 ever, a great amount of literature about niche  
2 requirements of competing species has been  
3 published for several taxa, reporting differences  
4 in three basic axes of the species niche: (i)  
5 trophic, including differences in prey type (e.g.,  
6 Labropoulou and Eleftheriou, 2005) or prey  
7 size (e.g., García and Arroyo, 2005), (ii) spa-  
8 tial, including differences in habitat (e.g., Hart,  
9 2003) or micro-habitat selection (e.g., Langk-  
10 ilde and Shine, 2004), (iii) temporal, including  
11 daily (e.g., Holm and Edney, 1973) or seasonal  
12 differences (e.g., Schuett et al., 2005) in their  
13 activity patterns. Segregation may occur along  
14 any of these niche dimensions, or some combi-  
15 nation of them (e.g., Loveridge and Macdonald,  
16 2003) and, thus, allow resource partitioning and  
17 the ecological coexistence of species.

18 Closely related species are supposed to com-  
19 pete intensely for resources in areas where their  
20 distributions overlap. Because they are recent  
21 descendants of a common ancestor, their current  
22 niches are the product of niche divergence and  
23 should be much overlapped (Wiens and Gra-  
24 ham, 2005). During the Quaternary ice ages,  
25 new species arose from the geographic separa-  
26 tion of the ancestral species in isolated refu-  
27 gia. Currently, when these largely allopatric  
28 new species meet at the limits of their parap-  
29 atric ranges, they are likely to display interspe-  
30 cific competition (Hewitt, 1996). Furthermore  
31 in contact zones, closely related species usu-  
32 ally experience gene flow as a result of hy-  
33 bridization processes (Howard et al., 2003). Tra-  
34 ditionally, natural hybridization has been in-  
35 terpreted as a mechanism to complete specia-  
36 tion processes (Mayr, 1963). In fact, the rein-  
37 forcement hypothesis predicts the development  
38 of pre-zygotic barriers for avoiding hybrids  
39 with reduced biological fitness when closely re-  
40 lated species come into contact (reviewed by  
41 Servedio and Noor, 2003). Nevertheless, hy-  
42 bridization is also interpreted as a new geno-  
43 type generator process, because from the com-  
44 bination of two parental genomes could result  
45 the foundation of new hybrid species, or as  
46 a mechanism allowing range expansion in un-  
47

48 favourable habitats, because introgression may  
49 lead to the transfer of adaptations from one  
50 taxon into another (Arnold, 1997; Burke and  
51 Arnold, 2001). In hybrid zones, interspecific  
52 competition among the different forms should  
53 be very strong and niche segregation should al-  
54 low coexistence (e.g., Heller, 1971; Gannon and  
55 Rácz, 2006) and the hybrids to achieve isolation  
56 from parental species (Arnold, 1997, Burke and  
57 Arnold, 2001).

58 European vipers are a good model to study  
59 the effects of competitive interactions and  
60 niche segregation mechanisms in contact zones.  
61 These vipers are a monophyletic group that  
62 evolved during the Quaternary ice ages un-  
63 der a probable allopatric speciation process  
64 (Lenk et al., 2001; Garrigues et al., 2005) and,  
65 with the exception of the *ursinii* group, have  
66 similar ecological requirements (Saint-Girons,  
67 1975a). Their current ranges are mostly parap-  
68 atric, but there are several contact zones among  
69 distributions of the different species (Saint-  
70 Girons, 1980a) where interspecific competition  
71 has been suggested to play an important role  
72 (Saint-Girons, 1975b, 1980a; Duguy, Martínez-  
73 Rica and Saint-Girons, 1979; Bea, 1985; Mon-  
74 ney, 1996; Luiselli, 2006; Luiselli, Filippi and  
75 Di Lena, 2007). Indeed, studies carried out  
76 in contact zones have reported spatial niche  
77 segregation among species in contact zones  
78 (Saint-Girons, 1975b; Duguy, Martínez-Rica  
79 and Saint-Girons, 1979; Bea, 1985; Monney,  
80 1996; Brito and Crespo, 2002; Luiselli, 2006;  
81 Luiselli, Filippi and Di Lena, 2007). How-  
82 ever quantitative studies were only developed  
83 in contact zones among phylogenetically dis-  
84 tant species (e.g., Saint-Girons, 1975b; Monney,  
85 1996; Brito and Crespo, 2002; Luiselli, Filippi  
86 and Di Lena, 2007), thus the role of interspecific  
87 competition and niche segregation mechanisms  
88 on closely related species, that could be inter-  
89 breeding, is still unknown.

90 In the Iberian Peninsula there are three para-  
91 patric viper species, two sibling Mediterranean,  
92 *V. aspis* and *V. latastei*, and one phylogenet-  
93 ically distant Euro-Siberian, *V. seoanei* (Lenk  
94

et al., 2001; Garrigues et al., 2005), which have several contact zones along their distribution areas. An allopatric distribution pattern at regional scale is found for the phylogenetically distantly-related pairs, *aspis-seoanei* and *latastei-seoanei*, whereas sympatry was suggested for the closely-related *aspis-latastei* in six areas of the Iberian Peninsula (Martínez-Freiría et al., 2009). In one of them, the high course of Ebro river (hereafter High Ebro), sympatry was observed in an area of 8 km<sup>2</sup> along the middle course of the rivers Rudrón and Sedanillo (Martínez-Freiría, Brito and Lizana, 2006a) and ecological niche-based models identified a gradual transitional environmental area of 76 km<sup>2</sup> where *V. aspis* and *V. latastei* could potentially be found together (Martínez-Freiría et al., 2008). Both species converge morphologically and specimens with intermediate characteristics were found in the sympatry area (Martínez-Freiría, Brito and Lizana, 2006a; Martínez-Freiría et al., 2009). Analyses of microsatellite variation in 210 specimens revealed the existence of gene flow between both species and that morphologically intermediate vipers correspond mostly to hybrids (authors, unpub. data). Moreover, cannibalism among vipers was detected (Martínez-Freiría, Brito and Lizana, 2006b), an aberrant behaviour in European vipers, which suggests the occurrence of strong competitive interactions.

In this study, the ecological traits of *V. aspis*, *V. latastei*, and hybrids will be investigated in the contact zone of High Ebro under the scenarios of interspecific competition and niche segregation. The aims of this study are the comparison of: (1) diet preferences (composition and annual variation, feeding frequency and trophic niche breadth and overlap); (2) micro-habitat use (item preferences by season); (3) activity patterns (% of active individuals, actual movement rate and home range size for males); (4) reproductive traits (fecundity, reproductive effort, biometry, mortality and moulting traits of new-borns, and frequency of reproduction); and (5) demographic traits (relative

abundance and mortality) of vipers. Diet preferences, micro-habitat use, and activity patterns will determine niche preferences, where similarities among the three viper forms will show that resources are under competition and differences will show that resources are being segregated. Reproductive and demographic traits will provide information about population dynamics and competitive advantages among the three different forms. Moreover, reproductive and demographic traits of hybrids will allow inferring their performance in the hybrid zone.

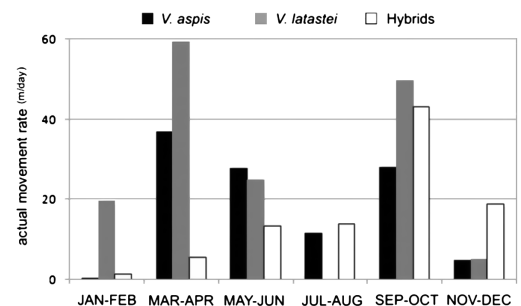
## Material and methods

### Study area

The study was conducted in the hybrid zone among *V. aspis* and *V. latastei* of the High course of Ebro river in northern Spain (fig. 1) (latitude: N42°37.7' to N42°58.7'; longitude: W3°37.3' to W3°58.5'). The area coincides with the transition between Euro-Siberian and Mediterranean regions, and consists of flat calcareous plateaus with deep valleys and steep canyons excavated by the Ebro River and its tributaries Rudrón and Sedanillo Rivers. Altitude ranges from 621 to 1150 m.a.s.l. Climate is sub-humid Mediterranean with Central European tendency. Average annual precipitation ranges from 697 to 829 mm/year and average annual temperature ranges from 22.2 to 24.8°C (Hijmans et al., 2005). The most representative bioclimatic stage is the Supra-Mediterranean but there are also elements of the Mountain stage of the Euro-Siberian region (for details see Martínez-Freiría, Brito and Lizana, 2006a).

### Field data

Data for this study were obtained through a combination of six distinct methodologies: (1) Random visual encounter surveys based on UTM 1 × 1 km squares, performed



**Figure 1.** Actual movement rate (AMR) by two month periods for males of *V. aspis*, *V. latastei* and hybrids in the High Ebro.

throughout the study area between March 2004 and October 2007. Vipers were captured by hand, photographed, counted for pholidosis, measured (mm), weighed, sexed, checked for sexual condition, and forced to defecate. Pseudo-replication was avoided by using individual identification (reference photographs and scale clipping). Stomach contents in live specimens were detected by palpation and vipers were not forced to regurgitate. A total of 303 specimens were used to study reproductive and diet traits. (2) Road sampling surveys, performed throughout the study area between March 2004 and October 2007. Road-killed vipers were photographed, counted for pholidosis, measured, sexed, and checked for sexual condition before dissection, in which digestive and reproductive organs, and embryos in the case of pregnant females, were extracted. Embryos were also counted for pholidosis, measured, and weighed. A total of 158 specimens were used to study reproduction, mortality, and diet traits. (3) Captive maintenance of pregnant females, performed during the last 20 days of gestation at controlled conditions in glass terrariums (560 × 300 × 300 mm). A total of 13 pregnant females were measured and weighed before and after giving birth. Date of parturition was noted and newborns were photographed, counted for pholidosis, measured, and weighed. New-borns were retained in captivity under controlled temperature and humidity conditions during the two days after birth. Data were used to study fecundity, biometric and moulting traits of new-borns. (4) Standardized transects, performed in five areas of the central part of the sympatry area (total length of 18.85 km) from March to October 2005 (avg. 4.05 transects by month). A total of 108 specimens were individually identified by clipping a unique combination of ventral scales (Blanchard and Finster, 1933) and used to determine the relative abundance of each of the three forms. (5) Radio-tracking surveys, performed on ten adult males (SVL between 440 and 505 mm) in the sympatry area from February 2006 to June 2007. Places occupied by these males were used to study microhabitat selection and activity patterns. (6) Ad hoc field observations, collected from March 2004 to October 2007, included assorted behaviours such as mating ( $n = 2$ ) and other reproductive behaviours (male combats or mate guarding,  $n = 35$ ) and selection of microhabitats and time periods for mating ( $n = 37$ ), gestation ( $n = 20$ ), hibernation ( $n = 91$ ) and moulting (table 1). These observations were used to complement data on reproductive, microhabitat, and activity patterns for the three forms.

Specimens were classified as *V. aspis*, *V. latastei*, or hybrids between both species according to a combination of morphological characters and analyses of microsatellite variation (authors; unpub. data). The morphological characters included snout elevation, number of apical scales, shape of the dorsal stripe, and number of dorsal markings (see Martínez-Freiría, Brito and Lizana, 2006a for details). The total dataset included 188 *V. aspis* (83 females and 105 males), 180 *V. latastei* (75 females and 105 males), and 93 hybrids (40 females and 53 males) (table 1).

### Radio-transmitters implantation

Two types of radio-transmitters were used: 3 Model PD-2 and 7 Model Holohil PD-2H, Holohil Systems Ltd. (Carp, Canada). Each model weighed 4 and 3 g, respectively, and represented on average 3.99% (range = 2.97-4.69%) of the vipers' body mass. Subcutaneous surgical implantation followed the procedures described by Weatherhead and Anderka (1984). Transmitters had a frequency range of 149.000-149.999 MHz and each type a battery life of 32 and 24 weeks, respectively.

Males were monitored between February 2006 and June 2007, which gave a total of 1669 observations: 629 for four males of *V. aspis*, 488 for three males of *V. latastei*, and 552 for three males of hybrids. Average length of monitoring was 5.5 months for *V. aspis* (range = 4-8), 5.3 months for *V. latastei* (range = 4-6) and 7.5 months for hybrids (range = 6-9). Males were located on average 10.8 days per month (range 1-23) between 0600 and 2100, and three times a day (range 1-6), with a minimum interval of two hours between consecutive locations. To minimize disturbing the vipers, they were located at a reasonable distance and their behaviour recorded without disturbing them. Nevertheless, they were captured at least once per month to inspect their condition.

### Diet preferences analyses

Whenever possible, undigested contents from vipers' stomachs and intestines were classified at the species level using identification keys (e.g., Teerink, 1991; Salvador, 1997). However, scales from lizards and hair from mammals have low morphological variation in species of the same genus, thus the determination at the species level of prey from the digestive tract was not possible for certain pairs of species with sympatric distributions in the study area. Such pairs included *Chalcides* spp., *Podarcis* spp., *Coronella* spp., and *Vipera* spp. in the case of reptiles (Pleguezuelos, Márquez and Lizana, 2002), and *Sorex* spp., *Microtus* spp., and *Apodemus* spp. in the case of mammals (Palomo and Gisbert, 2002). Preys were pooled in four categories: Invertebrates, Reptiles, Mammals-Soricomorpha, and Mammals-Rodentia.

Sexual and ontogenetic differences in diet composition were tested. Ontogenetic changes after 300 mm of snout-vent length (SVL) are common in *V. aspis* and *V. latastei* (Saint-Girons, 1980b; Luiselli and Agrimi, 1991; Santos et al., 2007). Data from dissected specimens from High Ebro suggested that sexual maturation is attained at this size (see below Reproductive traits analyses). Therefore, specimens were analysed with a separation between sexually immature and mature specimens (>300 mm SVL). Analyses among forms were done using  $\chi^2$  tests diet composition comparisons, annual variation, and feeding frequency. Trophic niche breadth and overlap were determined using Levin's and Pianka's indices, respectively.

**Table 1.** Diet, micro-habitat, activity and reproductive traits of *V. aspis*, *V. latastei* and hybrids in the High Ebro.

|                           | <i>V. aspis</i> | <i>n</i> | <i>V. latastei</i> | <i>n</i> | Hybrids   | <i>n</i> |
|---------------------------|-----------------|----------|--------------------|----------|-----------|----------|
| Diet preferences          |                 | 181      |                    | 172      |           | 85       |
| Composition (%)           |                 | 37       |                    | 48       |           | 28       |
| Mollusca                  | 0.00            |          | 2.17               |          | 3.57      |          |
| Coleoptera                | 0.00            |          | 4.35               |          | 0.00      |          |
| Invertebrates             | 0.00            |          | 4.17               |          | 3.57      |          |
| <i>Chalcides</i>          | 5.41            |          | 6.52               |          | 14.29     |          |
| <i>Podarcis</i>           | 0.00            |          | 2.17               |          | 0.00      |          |
| <i>Coronella</i>          | 0.00            |          | 2.17               |          | 0.00      |          |
| <i>Vipera</i>             | 2.70            |          | 2.17               |          | 0.00      |          |
| Reptiles                  | 8.11            |          | 14.58              |          | 10.71     |          |
| <i>Sorex</i>              | 8.11            |          | 13.04              |          | 10.71     |          |
| <i>Crocidura</i>          | 8.11            |          | 15.22              |          | 14.29     |          |
| Soricomorpha              | 16.22           |          | 20.83              |          | 17.86     |          |
| <i>Microtus</i>           | 43.24           |          | 32.61              |          | 42.86     |          |
| <i>Apodemus</i>           | 32.43           |          | 17.39              |          | 25.00     |          |
| Rodentia                  | 75.68           |          | 60.42              |          | 67.86     |          |
| Mammals                   | 91.89           |          | 81.25              |          | 85.71     |          |
| Niche breadth             |                 |          |                    |          |           |          |
| Levin's index             | 0.22            |          | 0.44               |          | 0.33      |          |
| Niche overlap             |                 |          |                    |          |           |          |
| Pianka' index             |                 |          |                    |          |           |          |
| <i>V. aspis</i>           | –               |          | 0.93               |          | 0.99      |          |
| <i>V. latastei</i>        | 0.93            |          | –                  |          | 0.95      |          |
| Hybrids                   | 0.99            |          | 0.95               |          | –         |          |
| Feeding frequency (%)     | 19.68*          | 181      | 26.67              | 172      | 30.11*    | 85       |
| Annual variation (%)      |                 | 181      |                    | 172      |           | 85       |
| Spring                    |                 | 13       |                    | 25       |           | 12       |
| Invertebrates             | 0.00            |          | 12.00              |          | 0.00      |          |
| Reptiles                  | 0.00            |          | 16.00              |          | 25.00     |          |
| Soricomorpha              | 30.77           |          | 32.00              |          | 8.33      |          |
| Rodentia                  | 69.23           |          | 40.00              |          | 66.67     |          |
| Summer                    |                 | 16       |                    | 14       |           | 12       |
| Invertebrates             | 0               |          | 0.00               |          | 8.33      |          |
| Reptiles                  | 12.5            |          | 14.29              |          | 8.33      |          |
| Soricomorpha              | 12.5            |          | 21.43              |          | 25.00     |          |
| Rodentia                  | 75              |          | 64.29              |          | 58.33     |          |
| Autumn                    |                 | 8        |                    | 6        |           | 7        |
| Invertebrates             | 0.00            |          | 0.00               |          | 0.00      |          |
| Reptiles                  | 12.50           |          | 0.00               |          | 0.00      |          |
| Soricomorpha              | 0.00            |          | 33.33              |          | 42.86     |          |
| Rodentia                  | 87.50           |          | 66.67              |          | 57.14     |          |
| Feeding period            | Mar.-Nov.       |          | Mar.-Oct.          |          | Apr.-Oct. |          |
| Micro-habitat preferences |                 |          |                    |          |           |          |
| Total presences           | 218             |          | 180                |          | 196       |          |
| Spring                    |                 | 4        |                    | 3        |           | 3        |
| BUSHES ( $\beta$ )        | 0.06**          |          | 0.17*              |          | –         |          |
| ROCKS ( $\beta$ )         | 0.07***         |          | 0.27*              |          | 0.11*     |          |
| SLOPE ( $\beta$ )         | –               |          | –2.87              |          | –         |          |
| TREES ( $\beta$ )         | –               |          | 0.14               |          | 0.20*     |          |
| Constant                  | –2.28           |          | –9.87              |          | –5.41     |          |
| AUC                       | 0.89            |          | 0.99               |          | 0.99      |          |
| S.E.                      | 0.04            |          | 0.01               |          | 0.01      |          |

**Table 1.** (Continued).

|                                  | <i>V. aspis</i>        | <i>n</i> | <i>V. latastei</i>     | <i>n</i> | <i>Hybrids</i>         | <i>n</i> |
|----------------------------------|------------------------|----------|------------------------|----------|------------------------|----------|
| CCR                              |                        |          |                        |          |                        |          |
| % pres. training                 | 82.86                  |          | 95.00                  |          | 94.74                  |          |
| % abs. training                  | 88.57                  |          | 95.00                  |          | 89.47                  |          |
| % pres. test                     | 100.00                 |          | 71.43                  |          | 100.00                 |          |
| % abs. test                      | 66.67                  |          | 100.00                 |          | 84.62                  |          |
| Total presences                  | 47                     |          | 27                     |          | 51                     |          |
| Summer                           |                        | 3        |                        | 3        |                        | 2        |
| BUSHES ( $\beta$ )               | 0.12*                  |          | 0.10                   |          | 0.07*                  |          |
| MOSS ( $\beta$ )                 | –                      |          | 0.17                   |          | –                      |          |
| ROCKS ( $\beta$ )                | 0.08*                  |          | –                      |          | –                      |          |
| SOIL ( $\beta$ )                 | –                      |          | –                      |          | 0.24*                  |          |
| TREES ( $\beta$ )                | 0.13*                  |          | –                      |          | 0.05*                  |          |
| Constant                         | –7.46                  |          | –4.93                  |          | –4.45                  |          |
| AUC                              | 0.94                   |          | 0.98                   |          | 0.94                   |          |
| S.E.                             | 0.03                   |          | 0.02                   |          | 0.04                   |          |
| CCR                              |                        |          |                        |          |                        |          |
| % pres. training                 | 86.36                  |          | 91.70                  |          | 89.47                  |          |
| % abs. training                  | 81.82                  |          | 91.70                  |          | 84.21                  |          |
| % pres. test                     | 57.14                  |          | 59.00                  |          | 100.00                 |          |
| % abs. test                      | 71.43                  |          | 100.00                 |          | 66.67                  |          |
| Total presences                  | 29                     |          | 16                     |          | 25                     |          |
| Autumn                           |                        | 3        |                        | 3        |                        | 2        |
| BUSHES ( $\beta$ )               | 0.22*                  |          | 0.12*                  |          | 0.18                   |          |
| ROCKS ( $\beta$ )                | –                      |          | –                      |          | 0.13                   |          |
| SLOPE ( $\beta$ )                | 3.46*                  |          | 4.81**                 |          | –                      |          |
| TREES ( $\beta$ )                | 0.08**                 |          | –                      |          | –                      |          |
| Constant                         | –15.65                 |          | –14.32                 |          | –7.90                  |          |
| AUC                              | 0.97                   |          | 0.97                   |          | 0.99                   |          |
| S.E.                             | 0.02                   |          | 0.02                   |          | 0.01                   |          |
| CCR                              |                        |          |                        |          |                        |          |
| % pres. training                 | 92.00                  |          | 100.00                 |          | 92.90                  |          |
| % abs. training                  | 88.00                  |          | 90.90                  |          | 92.90                  |          |
| % pres. test                     | 62.50                  |          | 91.67                  |          | 100.00                 |          |
| % abs. test                      | 87.50                  |          | 75.00                  |          | 100.00                 |          |
| Total presences                  | 33                     |          | 45                     |          | 19                     |          |
| Activity                         |                        |          |                        |          |                        |          |
| Days with activity for males (%) |                        | 4        |                        | 3        |                        | 3        |
| Jan.-Feb.                        | 0                      |          | 16.67 (0.0-33.3)       |          | 36.6 (0.0-0.6)         |          |
| Mar.-Apr.                        | 65.80 (57.1-72.7)      |          | 83.36 (81.0-85.7)      |          | 80.74 (77.3-84.2)      |          |
| May.-Jun.                        | 74.65 (35.0-100)       |          | 86.36 (72.7-85.7)      |          | 68.7 (56.5-89.5)       |          |
| Jul.-Aug.                        | 69.52 (28.6-100)       |          | 0                      |          | 43.5 (40.0-47.0)       |          |
| Sep.-Oct.                        | 48.15 (30.0-65.2)      |          | 49.6 (4.3-68.4)        |          | 41.7 (40.0-43.5)       |          |
| Nov.-Dec.                        | 0                      |          | 12.5 (0.0-5.0)         |          | 3.3 (0-10.0)           |          |
| Moulting periods                 |                        | 83       |                        | 75       |                        | 40       |
| Females                          | Apr.-Jun.<br>Aug.-Sep. |          | Apr.-Jun.<br>Aug.-Sep. |          | Apr.-Jun.<br>Aug.-Sep. |          |
| Males                            | Mar.-May.<br>Aug.-Oct. | 105      | Mar.-May.<br>Aug.-Oct. | 105      | Mar.-May.<br>Aug.-Oct. | 53       |
| Annually covered distance (m)    |                        | 4        |                        | 3        |                        | 3        |
| Avg.                             | 1033.4                 |          | 1295.2                 |          | 1331.33                |          |
| Min.                             | 253.6                  |          | 830.8                  |          | 593.6                  |          |
| Max.                             | 1910.8                 |          | 2283.0                 |          | 2070.0                 |          |

Table 1. (Continued).

|  | <i>V. aspis</i>       | <i>n</i> | <i>V. latastei</i>    | <i>n</i> | Hybrids        | <i>n</i> |
|--|-----------------------|----------|-----------------------|----------|----------------|----------|
| Reproductive traits                            |                       |          |                       |          |                |          |
| Detected mating period                         | April                 |          | April                 |          | March          |          |
| Sexual maturation (mm)                         |                       |          |                       |          |                |          |
| Females  |                       |          |                       |          |                |          |
| Smallest with embryos or developed follicles   | 341                   |          | 300                   |          | 414            |          |
| Smallest mating or with reproductive behaviour | 390                   |          | 470                   |          | 470            |          |
| Males  |                       |          |                       |          |                |          |
| Smallest mating or guarding females            | 395                   |          | 400                   |          | 440            |          |
| Date of parturition                            | late August-September |          | late August-September |          | late August    |          |
| Fecundity                                      |                       |          |                       |          |                |          |
| Avg. N° follicles                              | 7.39 (133 foll)       | 9        | 8.80 (88 foll)        | 5        | 8.80 (88 foll) | 5        |
| SD   | 2.56                  |          | 1.52                  |          | 1.44           |          |
| Avg. N° embryos                                | 5.75 (46 emb)         | 8        | 7.7 (77 emb)          | 10       | 5.6 (28 emb)   | 5        |
| SD   | 2.55                  |          | 2.45                  |          | 1.67           |          |
| Avg. reproductive effort                       | 0.59                  | 7        | 0.74                  | 8        | 0.79           | 3        |
| SD   | 0.24                  |          | 0.13                  |          | 0.28           |          |
| New-borns traits                               |                       |          |                       |          |                |          |
|  | 4 litters             | 28       | 7 litters             | 53       | 2 litters      | 12       |
| Avg. SVL (mm)                                  | 165.86***             |          | 152.42***             |          | 157.58***      |          |
| SD   | 11.09                 |          | 7.85                  |          | 19.36          |          |
| Avg. Body mass (g)                             | 5.28                  |          | 4.80                  |          | 4.75           |          |
| SD   | 0.88                  |          | 0.87                  |          | 1.28           |          |
| Avg. NSB                                       | 0.75*                 |          | 0.94*                 |          | 0.83*          |          |
| SD   | 0.44                  |          | 0.23                  |          | 0.39           |          |
| Avg. NMAP                                      | 0.61*                 |          | 0.81*                 |          | 0.50*          |          |
| SD   | 0.50                  |          | 0.39                  |          | 0.52           |          |
| Avg. NWFS                                      | 0.14**                |          | 0.00**                |          | 0.00**         |          |
| SD   | 0.36                  |          | 0.00                  |          | 0.00           |          |
| Frequency of reproduction (%)                  |                       |          |                       |          |                |          |
| 2004*  | 63.20                 | 19       | 65.00                 | 20       | 80.00          | 5        |
| 2005*  | 11.10                 | 18       | 20.00                 | 14       | 14.30          | 14       |
| 2006*  | 18.20                 | 11       | 35.30                 | 17       | 26.70          | 15       |
| Avg.   | 30.80                 | 48       | 40.10                 | 51       | 40.30          | 34       |
| SD   | 28.23                 |          | 22.88                 |          | 34.92          |          |

(\*significant differences for  $P = [0.05-0.005]$ ; \*\*significant differences for  $P = [0.005-0.001]$ ; \*\*\*significant differences for  $P < 0.001$ ). (AUC, area under the curve; SE, standard error; CCR, correct classification rate; pres., presences; abs., absences; Avg., average; Min., minimum; Max., maximum; SD: standard deviation; foll., follicles; emb., embryos; SVL: snout-vent length; NSB: number of still-borns; NMAP: number of moulted new-borns after parturition; NWFS: number of new-borns with fragments of skin after moult).

#### Microhabitat selection analyses

Microhabitat selection was inferred from the radio-tracked males (four *V. aspis*, three *V. latastei* and three hybrids). A total of 297 independent presence locations were recorded (except during the winter), of which 109 for *V. aspis*, 90 for *V. latastei*, and 98 for hybrids. It was also recorded the same amount of absence locations for the three forms, in places where specimens were never found. At each presence or absence locality, an area of 9 m<sup>2</sup> was delimited and characterised with nine uncorrelated variables (Pearson's  $r < 0.25$  in all cases): slope, % of bare soil, % of rocks, % of moss and substrate covering vegetation, % of bushes, and % of trees. Locations were grouped by form and season and then

analysed using logistic regression through a forward stepwise process ( $F$  to enter = 0.05). 75% and 25% of localities were used as training and test datasets, respectively. Models were validated using the Area Under the Curve (AUC) of a Receiver-operating curve (Liu et al., 2005).

#### Activity patterns analyses

Three indicators of activity were used: (1) % of active males. Vipers were considered as active when they were seen above the ground and not active when they were under rocks, in holes or inside dense bushes (Brito, 2003); (2) actual movement rate (AMR) for males was calculated as the sum of the distances moved divided by the number of days of

1 movement occurred in each interval; (3) home range size  
2 for males was calculated with KERNEL estimator (95% of  
3 probability) (Seaman and Powell, 1996). AMR and home  
4 range were estimated with Home Range Tools version 1.1  
5 for ArcGis 9.2 software. Comparisons between forms were  
6 performed using  $\chi^2$  tests.

### 7 *Reproductive traits analyses*

8 Because of the small sample size of males, statistical  
9 tests were done only for the new-born individuals and for  
10 the females. Potential fecundity was determined counting  
11 the number of enlarged follicles (>10 mm in diameter;  
12 Pleguezuelos et al., 2007), and calculating the average num-  
13 ber of enlarged follicles in road killed females. Effective fec-  
14 undity was determined as the average number of neonates  
15 by offspring and reproductive effort as the ratio of total off-  
16 spring body mass over body mass of the female just after  
17 parturition. Dissected road killed and captive pregnant fe-  
18 male datasets were used for calculating both fecundity pa-  
19 rameters, and also for estimating the smallest size for repro-  
20 duction by noting the smallest females with enlarged folli-  
21 cles or embryos.

22 Biometry, mortality, and moulting traits of newborns  
23 were determined using only data from captive pregnant fe-  
24 males. Biometric traits included comparisons of SVL and  
25 body mass. Mortality of newborns was calculated as the  
26 number of stillborns (NSB) after parturition. Moulting char-  
27 acteristics included comparisons regarding the number of  
28 moulted newborns after parturition (NMAP) and number  
29 of newborns with fragments of ancient skin after moult  
30 (NWFS). Usually, newborns immediately moult after birth,  
31 but occasionally they spend several days without moulting  
32 or maintaining fragments of the ancient skin (FMF, personal  
33 observation). Under controlled captivity conditions (temper-  
34 ature and humidity), abnormalities in moulting process, the  
35 maintenance of the ancient skin or fragments can be due  
36 to internal diseases in snakes, such as thyroid hypertrophy  
37 (AH-USA, 2009). Therefore, it was used as an indicator of  
38 the fitness of new-borns. Newborns were analysed grouped  
39 by litter and by form; comparisons were developed using  
40 one-way ANOVA analyses.

41 Frequency of reproduction was determined by calculat-  
42 ing the proportion of captured and road killed reproductive  
43 females by total adult females by year.  $\chi^2$  tests were per-  
44 formed among forms by years and by combining all years.  
45 Meteorological data (average precipitation, average temper-  
46 ature, average maximum temperature and average minimum  
47 temperature) for years 2003, 2004, and 2005 from adjacent  
48 meteorological stations (AEMET, 2008) were analysed with  
49  $\chi^2$  tests to evaluate relationships between variation among  
50 years in climate and proportion of reproductive females.

### 51 *Demographic traits analyses*

52 The relative abundance of vipers in the area of sympatry was  
53 compared using  $\chi^2$  tests by form, sex, and age. Mortality  
54 rates were estimated from a small section of 3.62 km located  
55 in the surroundings of the standardised transects. There  
56 were no significant differences ( $\chi^2 = 0.579$ ,  $P < 0.05$ )

57 in the number of road kill vipers by year and form for this  
58 section, thus all data were pooled. Comparisons among road  
59 kill vipers were performed using  $\chi^2$  tests by form, sex, and  
60 age.

## 61 **Results**

### 62 *Diet preferences*

63 There were no significant differences in diet  
64 composition between sexes of the same form,  
65 thus data for males and females were pooled.  
66 Comparing the three forms, there were no sig-  
67 nificant differences in diet composition when  
68 all specimens and only mature specimens were  
69 analysed (table 1). The Levin's index was rela-  
70 tively low (<0.440) and the Pianka's index was  
71 high (>0.933) for all forms (table 1).

72 Feeding frequency was not significantly dif-  
73 ferent among the three forms, but it was signifi-  
74 cantly different when *V. aspis* and hybrids were  
75 compared, being higher for the later. Annual  
76 variation in diet composition was not signifi-  
77 cantly different among seasons for each form  
78 and among the three forms by season. How-  
79 ever, there were trends for seasonal differences  
80 in prey consumption: (1) reptiles were mainly  
81 preyed upon in the summer by *V. aspis* and in  
82 the spring by *V. latastei* and hybrids, whereas  
83 Soricomorpha mammals were mostly consumed  
84 in the spring by parental species and in the sum-  
85 mer and autumn by hybrids (table 1); (2) *V. aspis*  
86 preyed more frequently upon Rodentia mam-  
87 mals in the summer and autumn than the other  
88 forms; (3) The main feeding season for *V. as-*  
89 *pis* and *V. latastei* occurred in the summer and  
90 the spring, respectively. Observed feeding pe-  
91 riod was one and two months longer in *V. as-*  
92 *pis* than in *V. latastei* and hybrids, respectively  
93 (table 1).

### 94 *Microhabitat selection*

95 Logistic regression models had high AUC  
96 (>0.890) and correct classification rates of  
97 training samples (>82%) but lower correct clas-  
98 sification of test samples (>57%), indicating



high accuracy but limited prediction capacity (table 1). Models identified variables % of bushes, % of rocks and % of trees as significantly related to the microhabitats selected by male vipers (table 1). Two of them, % of bushes and % of rocks, were linked to the selection of the three forms: males of all forms compete for areas with high amount of rocks in the spring and bushes in the summer and autumn. Moreover, several variables were related to the microhabitats selected by pairs of forms by season: (1) *V. aspis* and *V. latastei* selected areas with high amount of bushes in the spring and high slopes in the autumn; (2) *V. aspis* and hybrids selected areas with high amount of trees in summer; (3) *V. latastei* and hybrids selected areas with high amount of trees in spring. However, there were specific differences in microhabitat selection among males by season: (1) in the spring *V. latastei* selected areas with lower slope; (2) in the summer *V. aspis* used areas with high percentage of rock cover, *V. latastei* with mosses and hybrids with bare soil; (3) in the autumn *V. aspis* and hybrids occurred in areas with high percentage of tree and rock cover, respectively.

Moreover, field observations suggested the use of different places for hibernation and female's gestation. *V. aspis* was recorded hibernating in cooler places, with denser vegetation, closer to rivers or other humid areas than *V. latastei*, whereas the hybrids hibernated in places with intermediate characteristics. However, a hibernaculum with specimens of the three forms was found and consisted of a large crack in rock with sparse vegetation, located in the base of the plateau at 830 m of altitude. Gestation places for female *V. aspis* were also cooler than those used by female *V. latastei*, whereas hybrid reproductive females were found in places with intermediate characteristics.

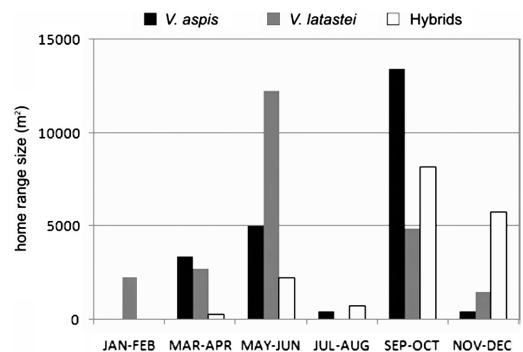
### Activity patterns

There were no significant differences among percentages of active males of the three forms.

However, there was a trend for a shorter period of activity in male *V. aspis* (table 1). Both radio-tracking and field observations at the beginning and end of the active season suggested that male *V. aspis* had a larger period of hibernation than *V. latastei* and hybrid males. Although there were no significant differences in the percentage of active males, males of *V. latastei* were mostly inactive during the summer (table 1); this was further corroborated by field observations.

There were significant differences in average actual movement rates (AMR) among males of three forms ( $\chi^2 = 104.37$ ,  $df = 10$ ,  $P = 0.001$ ). *V. aspis* and *V. latastei* had high AMR in March-April and a second peak in September-October, which was the highest for hybrids (fig. 1). *V. latastei* apparently does not move in July-August. Both *V. latastei* and hybrids exhibited low AMR in January-February and November-December, respectively.

There were significant differences in average home range size ( $\chi^2 = 20718.43$ ,  $df = 8$ ,  $P = 0.001$ ) among males of three forms throughout the annual cycle (fig. 2). The three forms exhibited peaks in the spring and autumn, but home range size oscillated throughout the year according to forms: (1) the largest sizes occurred in May-June for *V. latastei* but in September-October for *V. aspis* and hybrids; (2) relatively high values were observed in January-February and November-December in *V. latastei* and hybrids, respectively.



**Figure 2.** Home range sizes by two month periods for males of *V. aspis*, *V. latastei* and hybrids in the High Ebro.

1 Similar moulting periods were detected for  
2 147 and 161 adult females and males, respec-  
3 tively (table 1). Moulting periods for males oc-  
4 cur earlier in spring and later in summer-autumn  
5 than moulting periods for females.

### 7 *Reproductive traits*

8 Mating and copulatory behaviours were de-  
9 tected in the spring for the three forms (ta-  
10 ble 1). However, hybrid, interspecific, and  
11 hybrid-parental species pairs of males and fe-  
12 males were also detected during September and  
13 October, which suggested the occurrence of an  
14 autumn mating season for the three forms. Esti-  
15 mated minimum sexual maturation was smaller  
16 in both males and females of *V. aspis* and *V.*  
17 *latastei* but larger for hybrids (table 1). Date of  
18 parturition was similar among the three forms  
19 (table 1), occurring in late summer.

20 Potential and effective fecundity were differ-  
21 ent among *V. aspis* and *V. latastei*, the later pro-  
22 ducing more follicles and embryos. The number  
23 of follicles and embryos of hybrids was smaller  
24 than of *V. latastei* and *V. aspis*, respectively (ta-  
25 ble 1). Reproductive effort of *V. latastei* and hy-  
26 brids was similar and higher than in *V. aspis*.  
27 Analyses of the 10<sup>th</sup> percentile superior of fe-  
28 male's SVL shows that differences in fecundity  
29 and reproductive effort between forms are not  
30 size related ( $\chi^2 = 0.26$ ,  $df = 4$ ,  $P = 0.992$ ).

31 Significant differences in new-borns traits  
32 were only obtained when new-borns biomet-  
33 ric, mortality, and moulting traits were analysed  
34 pooled by form, without differing among litters.  
35 There were significant differences in new-borns  
36 SVL's of each form: *V. aspis* were larger and  
37 heavier than *V. latastei* whereas hybrids were  
38 intermediate. There were also significant differ-  
39 ences concerning mortality and moulting traits  
40 of new-borns: *V. aspis* had the lowest NSB and  
41 highest NWFS, *V. latastei* had the highest NSB  
42 and NMAP and hybrids had the lowest NMAP  
43 (table 1).

44 There were significant differences in the pro-  
45 portion of reproductive females by form among  
46 years but no significant differences among each  
47

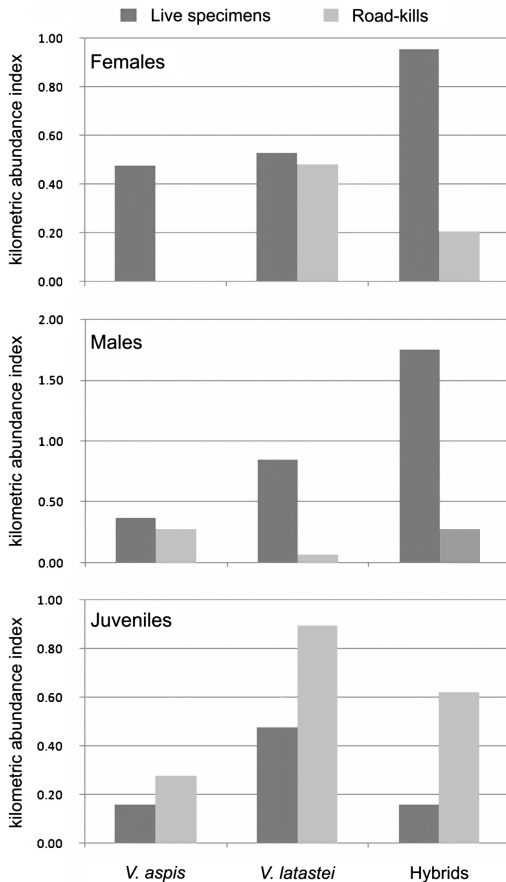
48 form in each year (table 1). Therefore, reproduc-  
49 tive cycles were similar among forms but vari-  
50 able among years. Significant differences ( $\chi^2 >$   
51 41.69,  $df = 22$ ,  $P < 0.05$ ) were found on aver-  
52 age temperature and maximum temperature for  
53 the period 2003-2005. 2003 was exceptionally  
54 warmer and that should have allowed a shorter  
55 hibernation period for the vipers. The average of  
56 the proportion of reproductive females for the  
57 three years ranged between 30 to 40%, which  
58 indicates a triennial reproductive cycle for the  
59 three forms.

### 61 *Demographic traits*

62 The relative abundance of forms by sexes and  
63 ages in the field was significantly different  
64 ( $\chi^2 = 9.54$ ,  $df = 4$ ,  $P < 0.05$ ): both male and  
65 female adult hybrids were more abundant than  
66 parental forms in the central part of the contact  
67 zone (fig. 3), whereas the most abundant juve-  
68 niles were of *V. latastei*. There was a trend for  
69 overall significant differences in road mortality  
70 among the three forms ( $\chi^2 = 9.42$ ,  $df = 4$ ,  
71  $P = 0.051$ ): female and juvenile *V. latastei*  
72 and juvenile hybrids had high road-mortality  
73 (fig. 3).

### 74 **Discussion**

75 This work revealed some patterns about the  
76 ecology of *V. aspis*, *V. latastei*, and hybrids  
77 in the contact zone of the High Ebro. Simi-  
78 larities in diet and microhabitat preferences of  
79 the three forms show that interspecific compe-  
80 tition has a major role in the dynamics of this  
81 hybrid zone. Differences in microhabitat use  
82 and activity patterns suggest the occurrence of  
83 niche segregation in spatial and temporal axes  
84 which should be relaxing competitive interac-  
85 tions among vipers. Nevertheless, differences  
86 in resource use could be apparently affecting  
87 fecundity, abundance, and mortality traits that  
88 should confer competitive advantages for one  
89 of the forms. Moreover, the hybrid population  
90  
91  
92  
93  
94



**Figure 3.** Kilometric indexes of abundance and road-mortality for females, males and juveniles of *V. aspis*, *V. latastei* and hybrids in the central region of the contact zone of High Ebro.

is well established as evidenced by their reproductive fitness and performance.

#### *Interspecific competition and niche partitioning*

Closely related species usually have similar ecological niches, thus interspecific competition should occur when they meet in contact (Wiens and Graham, 2005). At the High Ebro, the occurrence of interspecific competition was confirmed for the studied vipers mostly share the same prey and microhabitat preferences.

Diet partitioning is the most common mechanism for competition avoidance in many closely related species in contact (e.g., García and Arroyo, 2005), which includes many snake species

(Vitt, 1987; Luiselli, 2006). However, this is not the case among European vipers that have the same diet preferences, primarily preying upon small mammals and secondarily upon reptiles (Saint-Girons, 1980b; Luiselli and Agrimi, 1991; Bea et al., 1992; Brito, 2004; Santos et al., 2007; Canova and Gentili, 2008), even when they meet in contact (Saint-Girons, 1975b; Monney, 1996). Basically, they appear to prey on the most abundant prey available (Luiselli, 2006). Vipers from High Ebro follow the same pattern, having similar diet composition and presenting a total overlap of their trophic niches. In fact, prey availability studies confirm that the three forms mainly take the most abundant prey available: *Apodemus* mice (Lamosa, Martínez-Freiría and Pardavila, unpub. data).

Therefore, the three forms are competing intensely for the same prey items, but several factors could be minimizing competition. First, the great density of small mammals found in the area of sympatry in contrast with the allopatric areas (Lamosa, Martínez-Freiría and Pardavila, unpub. data). Furthermore, similar results were reported between sympatric *V. aspis* and *V. berus* in Switzerland (Monney, 1996): syntopy is possible in an area with a high density of micro-mammals. Therefore, if prey is not scarce, interspecific competition would be reduced.

Secondly, differences in the timing for eating might also reduce intense competition. In the High Ebro, the duration of the feeding period and the feeding frequency among vipers were quite different: *V. aspis* had the longest feeding period and the lowest feeding frequency, whereas hybrids had the shortest feeding period and the highest feeding frequency. *V. latastei* have intermediate values for both feeding period and frequency. Moreover, the main feeding season was different among forms: *V. aspis* and *V. latastei* consume nearly 50% of their preys in the summer and spring, respectively, whereas hybrids consume the same amount of prey in the two seasons. In France, similar results were found among *V. aspis* and *V. berus* in

1 the sympatry area of the Atlantic Loire (Saint-  
2 Giron, 1975b). These differences could be al-  
3 lowing temporal diet partitioning and, thus, re-  
4 ducing interspecific competition.

5 Finally, a trend for *V. aspis* being specialized  
6 in rodents might indicate trophic segregation.  
7 Particular differences in prey consumption were  
8 also reported in sympatric *V. aspis* and *V. berus*  
9 (Saint-Giron, 1975b). At the High Ebro, differ-  
10 ences in prey consumption are noticeable when  
11 the annual variation in prey consumption is con-  
12 sidered and probably is related to differences  
13 in prey availability in the microhabitats used  
14 throughout the annual cycle (e.g., Brito, 2003,  
15 2004; Agrimi and Luiselli, 1992). Therefore, at  
16 the High Ebro, competition on the trophic axis  
17 should be very strong, since the three forms con-  
18 sume the same prey. However, prey abundance  
19 and some degree of diet partitioning throughout  
20 the annual cycle could reduce competitive inter-  
21 actions.

22 Microhabitat selection has been confirmed as  
23 the major axis for niche partitioning among Eu-  
24 ropean vipers in contact (Luiselli, 2006). For  
25 example, *V. aspis* use warmer and dryer mi-  
26 crohabitats than *V. berus* in French and Swiss  
27 contact zones (Saint-Giron, 1975b; Monney,  
28 1996). In the Pyrenees, similar results were re-  
29 ported in the Spanish contact zone among *V. as-*  
30 *pis* and *V. latastei*: the former occurred in hu-  
31 mid micro-habitats, north exposed, whereas the  
32 later occurred in south-facing dry microhabi-  
33 tats (Duguy, Martínez-Rica and Saint-Giron,  
34 1979). In contact zones, different microhabitat  
35 selection has been referred as due to the dif-  
36 ferent thermal needs and thermoregulatory abil-  
37 ities of the species, but also as mediated by  
38 interspecific competition (Saint-Giron, 1975a,  
39 1978, 1980a; Duguy, Martínez-Rica and Saint-  
40 Giron, 1979). Out-competition from a deter-  
41 minate spot through interference competi-  
42 tion has been documented in many species  
43 of reptiles (e.g., Langkilde and Shine, 2004)  
44 and mutual exclusion was referred as occur-  
45 ring among European vipers in contact zones  
46 (Duguy, Martínez-Rica and Saint-Giron, 1979;  
47

Saint-Giron, 1975b, 1980a; Bea, 1985). At the  
48 High Ebro, the three forms select similar items  
49 each season, thus there should be a strong com-  
50 petition for places with the same characteris-  
51 tics. Moreover, the observation of cannibalistic  
52 behaviours in *V. latastei* in captivity conditions  
53 (Martinez-Freiría, Brito and Lizana, 2006b) and  
54 the detection of fragments of vipers in gut con-  
55 tents of parental species (table 1), suggest the  
56 occurrence of out-competition. Therefore, in-  
57 terference competition could let out-competed  
58 vipers use different microhabitats. In fact, at  
59 the High Ebro there are microhabitat compo-  
60 nents that are selected only by one of the forms  
61 per season. For example, during the summer,  
62 which is the main feeding period for vipers (Bea  
63 et al., 1992; Brito, 2004), microhabitat selec-  
64 tion is quite different: *V. aspis* selects warmer  
65 (with rocks) microhabitats than those used by  
66 *V. latastei* (with moss), whereas microhabitats  
67 selected by the hybrids are intermediate (with  
68 soil). Male *V. aspis* and *V. latastei* in allopa-  
69 try were reported using fresh microhabitats dur-  
70 ing the summer (Moser, Graber and Freyvo-  
71 gel, 1984; Naulleau, Duguy and Saint-Giron,  
72 1998; Brito, 2003). At the High Ebro, sympatric  
73 male *V. aspis* are apparently out-competed to  
74 suboptimal microhabitats by the other forms.  
75 Moreover, field observations suggest the use of  
76 places with different characteristics for some  
77 crucial periods such as hibernation or females'  
78 gestation. Furthermore, these places are distinct  
79 from those used in areas of allopatry. For ex-  
80 ample, in the allopatric range of *V. aspis* in  
81 the High Ebro, pregnant females were detected  
82 in warmer places with low vegetation (authors,  
83 personal observation), whereas in the sympa-  
84 try area pregnant females were only detected  
85 in cool places with dense vegetation. However,  
86 both in allopatry and sympatry, pregnant fe-  
87 males of *V. latastei* were detected during gesta-  
88 tion in warmer places with low vegetation (au-  
89 thors, personal observation). This suggests that  
90 in sympatry, female *V. aspis* are out-competed  
91 to suboptimal places by the other forms. There-  
92 fore, interspecific competition seems to leading  
93  
94

1 to spatial partitioning among vipers at the High  
2 Ebro, which seems to be causing the use of sub-  
3 optimal places in *V. aspis*. Nevertheless, ther-  
4 moregulation studies are needed to determine  
5 thermal characteristics of the places used by the  
6 different viper forms.

7 Activity is the third niche axis in which sim-  
8 ilar ecological species in contact could differ  
9 to avoid competition (Ricklefs, 1998) and even  
10 interbreeding (Schuett et al., 2005). Studies in  
11 contact zones between *V. aspis* and *V. berus*  
12 have reported differences in the duration of the  
13 activity period and in the time for mating (Saint-  
14 Girons, 1975b; Monney, 1996). However, con-  
15 sidering that the species were at the limits of  
16 their extent of occurrence, these differences  
17 could also be due to different thermal needs and  
18 thermoregulation abilities (Saint-Girons, 1975a,  
19 1975b, 1978; Monney, 1996), instead of inter-  
20 specific competition. At the High Ebro, the du-  
21 ration of the hibernation period is quite dif-  
22 ferent among forms, being larger in *V. aspis*  
23 than in *V. latastei* and hybrids. Moreover, male  
24 movement rates and home range sizes increased  
25 in the spring and autumn, which coincide ex-  
26 actly with the two mating periods reported for  
27 *V. aspis* and *V. latastei* (Saint-Girons, 1957,  
28 1992; Brito, 2003; Pleguezuelos et al., 2007).  
29 At these times, males cover large distances  
30 searching for females to mate, thus increasing  
31 their home ranges (Naulleau, Duguay and Saint-  
32 Girons, 1998; Bonnet, Naulleau and Shine,  
33 1999; Brito, 2003). In allopatric populations,  
34 the mating period for *V. aspis* occurs predom-  
35 inantly in the spring and secondly in autumn,  
36 whereas for *V. latastei* occurs mainly in the au-  
37 tumn (Saint-Girons, 1957, 1992; Moser, Graber  
38 and Freyvogel, 1984; Brito, 2003; Pleguezuelos  
39 et al., 2007). At the High Ebro, field observa-  
40 tions detected the occurrence of a spring mating  
41 period and suggested the occurrence of an au-  
42 tumn mating period for the three forms; inter-  
43 specific couples were also found at these times  
44 (Martínez-Freiría, Brito and Lizana, 2006a).  
45 However, home ranges estimates for males sug-  
46 gest different periods for mating: *V. aspis* and

48 hybrids mainly should mate in the autumn,  
49 whereas *V. latastei* in the spring. Moreover, the  
50 values for the home range estimates at these  
51 times were different among the three viper  
52 forms and that could be mediated by interfe-  
53 rence competition. Therefore, vipers from the  
54 High Ebro probably mate in the spring and  
55 the autumn, but the use of space during mat-  
56 ing periods would be different among forms,  
57 which could reduce the occurrence of interspe-  
58 cific matings. Nevertheless, the role of interspe-  
59 cific competition in temporal niche segregation  
60 remains unclear because the thermal require-  
61 ments of the three forms are unknown.

### 62 *Competitive advantages*

63 Differences in resources use should have reper-  
64 cussions on reproductive and demographic traits  
65 of species in competition. Such differences may  
66 confer competitive advantages for one of the  
67 species in detriment of the others, for they af-  
68 fect life history traits and, thus, the dynamics  
69 of populations (Clutton-Brock, 1990). In fact,  
70 such differences have been reported among phy-  
71 logenetically distant *V. aspis* and *V. berus* in the  
72 French Atlantic-Loire and Swiss pre-Alps con-  
73 tact zones: *V. berus* reproduces more frequently  
74 but is more vulnerable to predation than *V. aspis*,  
75 which influences population dynamics in both  
76 contact zones (Saint-Girons, 1975b; Monney,  
77 1996). At the High Ebro, all forms exhibit some  
78 dietary differences but clearly segregate their  
79 spatial and temporal niches. Therefore, differ-  
80 ences in reproductive and demographic traits of  
81 vipers could be due to these niche differences.

82 Higher reproductive success may confer  
83 competitive advantage for one of the forms be-  
84 cause it affects population size (Clutton-Brock,  
85 1990). At the High Ebro, differences were found  
86 between *V. aspis* and *V. latastei* in the number,  
87 body mass, and body size of newborns. *V. aspis*  
88 produces fewer but larger and heavier newborns  
89 per litter than *V. latastei*, whereas hybrids have  
90 intermediate values for these traits. These differ-  
91 ences probably are not related to phylogenet-  
92 ically different reproductive strategies, given that  
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opposing patterns were reported in allopatric populations of *V. aspis* and *V. latastei*. Both of these species have similar fecundity and new-borns of *V. latastei* are larger and heavier than those of *V. aspis* (Saint-Girons and Naulleau, 1981; Pleguezuelos et al., 2007). Therefore, differences in fecundity traits among allopatric and sympatric (High Ebro) populations could be due to distinct use of resources that may be mediated by interspecific competition. For instance, optimal thermoregulatory conditions during gestation affect pregnant female's output (Lourdais et al., 2004), thus higher mortality rates or diseases in new-borns could be due to the use of sub-optimal thermoregulatory spots by females during pregnancy. At the High Ebro there were significant differences in mortality between forms and moulting traits of new-borns. *V. aspis* had more stillborn per litter and more newborns without moulting or maintaining fragments of ancient skin after parturition than *V. latastei*. Hybrids usually have intermediate values for these traits. Therefore, out-competition from optimal places could be promoting the use of sub-optimal thermoregulatory spots during pregnancy of *V. aspis* and hybrid females, and affecting their litters. Therefore, attending reproductive success of vipers from the High Ebro, *V. aspis* is apparently less competitive than *V. latastei* and hybrids are more competitive than *V. aspis* and less than *V. latastei*.

Differences in abundance and mortality may confer competitive advantages for one of the forms because they affect lifetime reproductive success (Clutton-Brock, 1990). At the High Ebro, there were significant differences in the abundance of the three forms: hybrids were more abundant than the parental forms. However, it contradicts results from fecundity, in which *V. latastei* should be the most abundant form, suggesting that other mechanisms influence population size of each form. Indeed, road-mortality is higher for male *V. aspis* and female *V. latastei* than for hybrids. Mortality on roads can be used as an indicator of predation risk because snakes are more vulnerable to

predators (including natural and anthropogenic) when they travel outside their normal home range (Bonnet, Naulleau and Shine, 1999). Differences in mortality due to thermoregulation abilities of *V. aspis* and *V. berus* were reported for the Atlantic Loire (Saint-Girons, 1975b). At the High Ebro, the periods of high mortality detected may be related to mating periods in males. During the mating season, the search for females increases the vulnerability of males to predation (Bonnet, Naulleau and Shine, 1999). In the study area, higher mortality period for males of *V. aspis* occur in the autumn when they increase their home ranges due to mating activities. Therefore, distinct thermal conditions and abilities, and differences in the use of space during mating periods could be related to different mortality rates of males.

Feeding after reproduction is essential for females because they invest body fat reserves on reproduction and during gestation they eat relatively little, and some may become completely anorexic (Saint-Girons, 1957). Females of European vipers are capital breeders, i.e., females need to accumulate body fat reserves to reach reproductive status (Bonnet et al., 1999). Consequently, reproduction costs, i.e., how female's investment on reproduction (FIR) affects their survival, have to be determined after parturition and also during the time expended to reach again the reproductive status (Bonnet et al., 1999). Body condition index (length-mass relationship) is a valid indicator of previous food intake rate and the size of energy reserves in snakes (Bonnet and Naulleau, 1994) and is associated with both costs and benefits that may influence mortality after reproduction. At the High Ebro, major mortality periods for female *V. latastei* correspond to late spring and late summer, during which they move away from their hibernation and gestation places to feeding areas (authors, personal observation). Body condition indices for most of road killed females were very low, which indicate that they were reproductive during the previous year in the case of road killed females in late spring, or

1 the present year in the case of road killed females in late summer. Despite the intermediate  
2 FIR of *V. latastei*, costs of reproduction in this  
3 form should be the highest due to high mortality  
4 of post-reproductive females. Probably, investments  
5 on reproduction, but also selection of gestation  
6 places and hibernation periods, might be affecting  
7 mortality rates of female *V. latastei*.

8 It was suggested that populations of *V. latastei*  
9 are contracting while *V. aspis* is expanding in  
10 northern Iberian Peninsula, due to interspecific  
11 competition (Pleguezuelos and Santos, 2002).  
12 However, the low reproductive success of *V. aspis*  
13 at the High Ebro suggests that it should not be  
14 able to expand southwards because it is less  
15 competitive than *V. latastei* and even the hybrid  
16 forms. Contrarily, the high female mortality  
17 of *V. latastei* suggests that it should not be  
18 able to expand northwards because it is less  
19 competitive than the other forms. Although sample  
20 size is small, both reproductive and demographic  
21 differences suggest a probable balance among the  
22 population dynamics of *V. aspis* and *V. latastei*  
23 at the High Ebro.

#### 24 Hybrid population

25 Endogenous selection (or environmental independent)  
26 is the major force implicated in the formation of  
27 tension zones (Barton and Hewitt, 1985), which  
28 is the most common model of hybrid zones (Arnold,  
29 1997; Burke and Arnold, 2001). Endogenous  
30 selection is due to chromosomal structural  
31 differences and recombination in hybrid genotypes  
32 (Arnold, 1997) and could result in lowered  
33 viability or fertility of hybrids (e.g., Barton and  
34 Hewitt, 1985; Szymura and Barton, 1991). Little  
35 is known about hybridization and selection  
36 mechanisms in contact zones among European  
37 vipers. The known cases of fertile hybrids  
38 between European vipers come from captivity  
39 where reproduction was forced (e.g., *V. ammodytes*  
40  $\times$  *V. aspis*, Obermayer, 1967; *V. aspis*  $\times$   
41 *V. seoanei*, Saint-Girons, 1990a, 1990b).  
42 Natural hybridization in contact zones among  
43 European vipers is anecdotal (Saint-Girons,  
44 1975b, 1980a; Monney, 1996) but the

45 existence of specimens with intermediate morphological  
46 traits in contact zones suggests the occurrence of  
47 hybridization (Zuffi et al., 2001; Brito et al.,  
48 2008). At the High Ebro, hybrids have intermediate  
49 values for reproductive traits but higher and lower  
50 for abundance and mortality than parental forms,  
51 respectively. Moreover, the high mortality rates  
52 for male *V. aspis* and female *V. latastei* suggests  
53 the occurrence of disequilibrium among sexes of  
54 parental forms, which could be favouring  
55 interspecific mates. Therefore, interspecific  
56 mates could be maintaining the hybrid population,  
57 which have an intermediate reproductive fitness  
58 but a good performance in the hybrid zone.  
59 Nevertheless, fitness of hybrid genotypes and  
60 allele frequencies distributions in the hybrid zone  
61 need further study to test these hypotheses.

#### 62 Conclusions and future research

63 This study's combination of different methodologies  
64 was appropriate to infer the ecological patterns  
65 of vipers from the High Ebro. In this hybrid  
66 zone, *V. aspis*, *V. latastei*, and hybrids seem  
67 to be competing intensely for resources. However,  
68 partition on the spatial and temporal axes of  
69 their niches could be reducing interspecific  
70 competition and allowing coexistence. The use  
71 of different resources might be mediated by  
72 inference competition, conferring reproductive  
73 and demographic advantages for *V. latastei*  
74 and hybrids, respectively. Reproductive fitness  
75 of hybrids is an intermediate between those  
76 of the two parent species, which suggest the  
77 occurrence of endogenous selection. Nevertheless  
78 comparative thermal and genetic studies are  
79 needed to elucidate the limiting factors for  
80 the three forms and to establish a model of the  
81 hybrid zone.

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