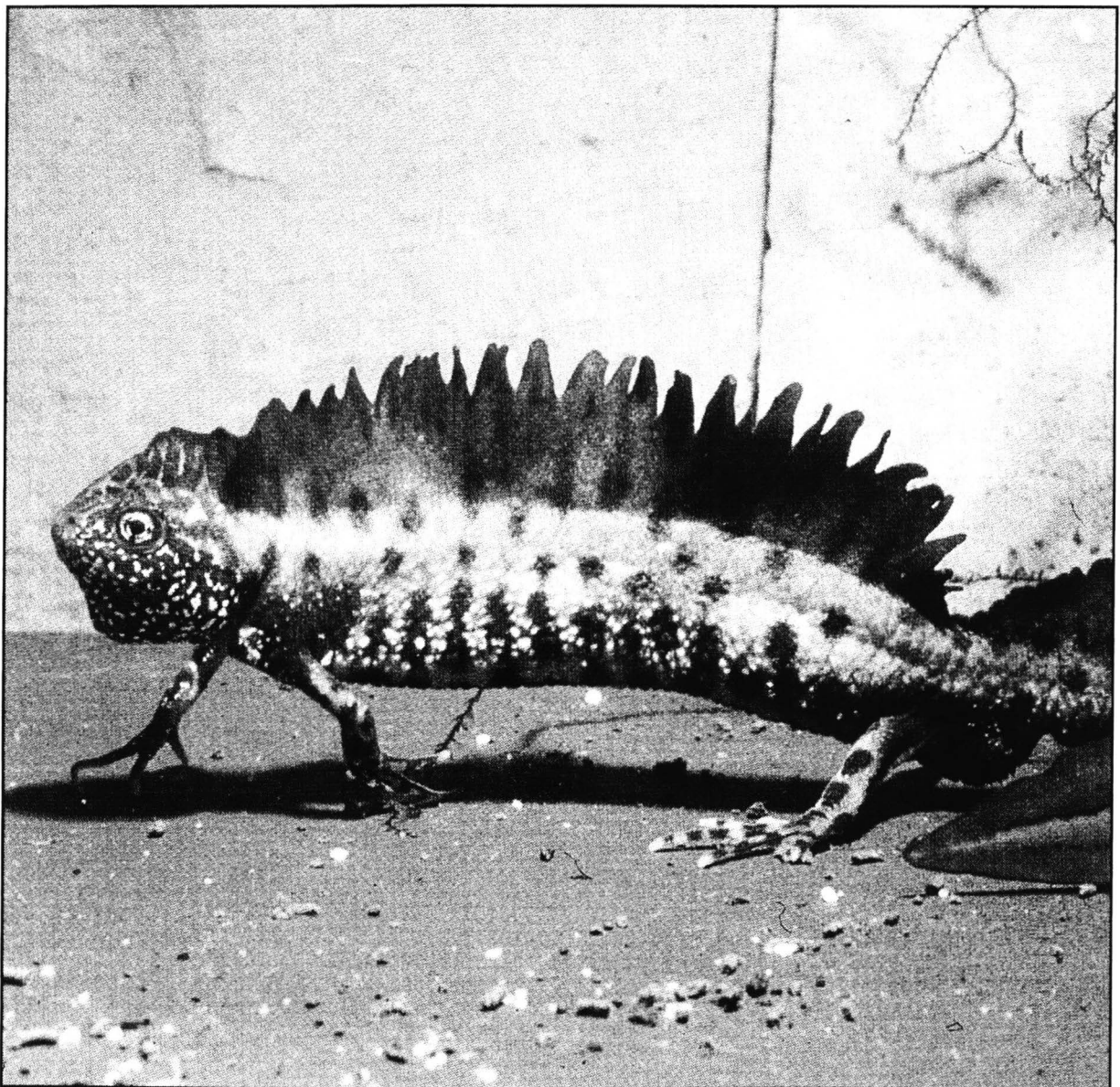


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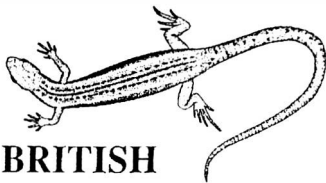
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FAECES AVOIDANCE BEHAVIOUR IN UNISEXUAL AND BISEXUAL GECKOS

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The unisexual gecko, *Lepidodactylus lugubris*, harbours fewer types of parasites and has lower prevalence of infection than does the bisexual gecko, *Hemidactylus frenatus*, or its bisexual parental species. Because many diseases and parasites are transmitted through faeces, we conducted a series of experiments to examine whether or not *L. lugubris* had a greater tendency to avoid faecal matter than *H. frenatus*. The research found that both species defecated away from their daytime hiding places. The unisexual geckos, when given a choice, picked hiding places that were surrounded by clean rather than contaminated sphagnum moss, or places that were sprayed with distilled water rather than faecal solution. The bisexuals also displayed a tendency to pick hiding places surrounded by uncontaminated sphagnum moss, but, unlike the unisexuals, did not avoid faecal solution. Neither species avoided dried faecal matter. These data support our hypothesis that the unisexual gecko, *L. lugubris*, exhibits more parasite avoidance behaviours than the bisexual gecko, *H. frenatus*.

INTRODUCTION

The unisexual gecko, *Lepidodactylus lugubris*, harbours fewer types of parasites and has a lower rate of parasitism than the bisexual gecko, *Hemidactylus frenatus*, both in Hawaii (Brown *et al.*, 1995) and throughout the Pacific Basin (Hanley, Volmer & Case, 1995). Lower parasitism rates are found for both endoparasites transmitted by the faecal-oral route and ectoparasites, like mites. Hanley, Fisher & Case (1995) found that when *L. lugubris* lived in sympatry with either of its parental species, *Lepidodactylus moestus* and *Lepidodactylus* undescribed species (Radtky *et al.*, 1995), the bisexual parental species had a higher prevalence of – and more intense – mite infestations than the unisexual hybrid. Moreover, in their experiments on mite exposure, they found that in both mixed and single species conditions, more of the bisexual geckos contracted a mite infection than the unisexuals. Brown *et al.* (1995) obtained similar findings on mite transfer between *L. lugubris* and *H. frenatus*.

Continuing comparisons between disease rates and parasite avoidance behaviours in unisexual and bisexual species are important for testing the Red Queen hypothesis of the evolution of sex. The Red Queen hypothesis states that in the long term, bisexual species have an advantage over unisexual species in adapting to new parasites or to old parasites with newly evolved means of transmission or virulence (Seger & Hamilton, 1988). In the short term, however, unisexual species may have reduced prevalence of parasites compared to the bisexual species with which it lives in sympatry (Brown *et al.*, 1995).

Host species should evolve not only efficient immunological systems against disease organisms but also behaviours that enable individuals to avoid becoming parasitized. For example, Atkinson & Van Riper III (1991) hypothesized that Hawaiian forest birds now sleep with tucked up bills and faces and a raised leg to

protect exposed skin from mosquito bites. These behaviours were not observed in endemic Hawaiian birds prior to the introduction of mosquitos that carried avian malaria and pox to the Hawaiian islands. Some species, such as chimpanzees, seek out and consume plants with compounds that kill off their parasites (Rodriguez & Wrangham, 1993), and recently Hemmes *et al.* (1995) reported that wood rats line their sleeping quarters with leaves that kill flea larvae. Many species also avoid contact with faecal matter. For example, jackdaws and some tits carry faeces away from their nests (Lorenz, 1970) and many animals defecate away from their normal living sites (Grier, 1984). Additionally, great tits avoid parasitized nest sites (Merila & Allander, 1995).

The following experiments examined faecal avoidance behaviour in the unisexual gecko, *L. lugubris*, and the bisexual gecko, *H. frenatus*. The experiments were designed to examine whether the presence of faeces influenced the selection of daytime hiding places and whether the species avoided defecating near their daytime hiding places. We hypothesized that the unisexual and bisexual species would differ in the number and types of their faecal avoidance behaviours which would account for some of the differences in intestinal parasitism rates displayed by the two species.

METHODS

The first series of experiments was conducted to determine whether geckos chose daytime hiding places that were surrounded by sphagnum moss contaminated with faeces, or free from faeces (uncontaminated). Twenty *L. lugubris* and 18 *H. frenatus* were used as subjects in Experiment 1. Aquaria (51 x 28 x 31 cm) served as testing chambers. A small platform (8.5 x 3.5 cm) was placed at each end of every aquarium. These platforms simulate the characteristics of naturally occurring hiding places and are readily used by wild-captured geckos. One platform was surrounded by

clean sphagnum moss and the other by sphagnum moss that had been used as bedding in an enclosure housing at least three geckos for a period of two months. Placement of moss as well as species of gecko were counterbalanced across aquaria. When an aquarium was reused, the uncontaminated/contaminated moss was placed on the opposite side and a different species of gecko was used as the subject (all aquaria were sanitized between experimental runs).

A gecko in an open bottle was placed in the middle of the aquarium (half way between the two platforms) in the afternoon. The next morning the position of the gecko was noted (i.e. in the bottle, on top of or inside a platform, or in the sphagnum moss). In addition, 10 of the above *L. lugubris* and nine of the *H. frenatus* were observed every 5 min for the first 90 min after they were placed in the aquarium. Observations were conducted to see whether the geckos used purely chemical cues in making their choices (in which case we hypothesized that they would not enter the contaminated moss) or tactile and chemical cues.

In Experiment 2, the platforms were surrounded by either dried faeces placed in an arc approximately 5 mm wide and 3 mm in depth or by nothing (34 *L. lugubris* and 28 *H. frenatus* were tested). In Experiment 3, the floor of the aquarium surrounding the platform was either sprayed with a solution of 15 ml ground dried faeces suspended in 300 ml distilled water or distilled water alone (50 *L. lugubris* and 52 *H. frenatus* were tested). The spray bottle containing the suspended faecal solution was vigorously shaken prior to each use. Gecko species and the placement of the stimuli were counterbalanced across the aquaria. All aquaria were sanitized prior to each use with a 1:1 bleach to water solution and were immediately wiped dry. Both experiments were conducted during the daytime. The geckos were placed in the aquaria between 8.00 and 9.00 am and positions of the geckos were noted 6 hrs later.

Experiment 4 examined whether or not geckos defecated near or away from their daytime hiding places. Ten *H. frenatus* (five females and five males) and 10 *L. lugubris* were housed individually in perspex enclosures (32 x 18 x 23 cm). One small platform was centred along the back of each enclosure. Geckos were housed in the enclosures for a period of four days. Systematic observations (12 per day) were made of where each gecko was located within the enclosure to determine its preferred daytime hiding place. At the end of the four day period, the geckos were removed. Measurements were obtained on where each gecko defecated in relation to the gecko's most frequent daytime hiding place.

RESULTS

In Experiment 1, more geckos were found on the sides of the aquaria surrounded by uncontaminated sphagnum moss (13/17 *L. lugubris* and 11/15 *H. frenatus*) than on the contaminated sides (Fig. 1).

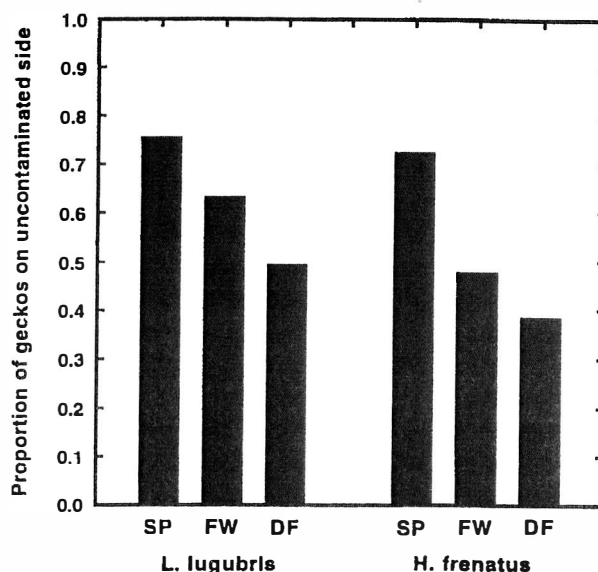


FIG. 1. Proportion of geckos that chose the uncontaminated side of the aquarium. SP refers to the geckos that were tested with sphagnum moss, FW to those tested with faeces mixed in distilled water, and DF to those tested with dry faeces.

When tested with χ^2 (corrected for continuity), the *L. lugubris* exhibited a significant preference for the uncontaminated moss and platform ($\chi^2 = 4.82$, $df=1$, $P < 0.05$), but *H. frenatus* did not ($\chi^2 = 3.33$, $df=1$, $0.05 < P < 0.10$), perhaps due to the small sample size. Six geckos (three *L. lugubris* and three *H. frenatus*) were found in the bottles or at the top of the aquarium; these animals were not included in the analysis.

Of the geckos which were examined in greater detail, one gecko of each species did not leave the bottle during the 90 min, five geckos moved to either the uncontaminated side ($n = 3$) or to the top of the aquaria ($n = 2$) and remained there, and 10 geckos explored the aquaria more thoroughly. Eight moved to the contaminated side of the enclosure and then alternated back and forth between the sides, and two geckos moved to the uncontaminated side and then alternated. Of these 10

TABLE 1. The behaviours geckos displayed when they were observed every 5 minutes for 90 minutes in Experiment 1. Bottled geckos were placed between two platforms, one surrounded by clean sphagnum moss (uncontaminated), the other by sphagnum moss that contained gecko faeces (contaminated).

Behaviour	Species of Gecko	
	<i>L. lugubris</i>	<i>H. frenatus</i>
Remained in bottle	1	1
Moved to an uncontaminated area and remained	4	1
Moved to the uncontaminated side and alternated sides	1	1
Moved to the contaminated side and alternated sides	3	5

geckos, eight were found the next morning on the uncontaminated side of the aquarium and two on the contaminated side (Table 1).

Neither *L. lugubris* nor *H. frenatus* showed a preference for a particular side of the aquaria when dry faeces were used as stimuli in Experiment 2. Half of the *L. lugubris* (17/34) and 61% (17/28) of the *H. frenatus* were found on the platforms surrounded by dried faecal matter after 6 hrs (Fig. 1). In contrast, when faecal matter was in solution with distilled water (Experiment 3), *L. lugubris* displayed a significant preference for the platform sprayed with distilled water (32/50; $\chi^2 = 3.94$, $df=1$, $P < 0.05$) over the platform sprayed with faecal solution (18/50). *H. frenatus* did not display this preference (25/52 chose distilled water; Fig. 1). However, the difference between species was not significant ($\chi^2 = 1.52$, $df=1$, $P > 0.05$).

In Experiment 4, both species of gecko defecated away from their preferred daytime hiding places. *L. lugubris* defecated an average 22.6 cm from their hiding places and *H. frenatus* 13.2 cm. The difference between species was not significant ($F_{1,17} = 1.5$, $P > 0.05$).

DISCUSSION

Both the unisexual and bisexual gecko species defecated away from their preferred daytime hiding places, and when given a choice, preferred hiding places that were surrounded by faeces-free sphagnum moss. The unisexuals also avoided areas where faeces mixed with water had been sprayed, but neither species avoided crossing areas of dried faecal matter. A similar pattern is observed in geckos that inhabit man-made structures. After 12 years of observing nocturnal geckos, the senior author has never observed faecal matter near their daytime hiding places. Faecal matter accumulates, however, on ledges such as window casings. Ledges are crossed by geckos at dusk when the animals are climbing up to feed. Avoidance of faecal matter has been reported in birds (Lorenz, 1970) but has not, as far as we know, been reported before in a lizard species.

If contact with fresh faeces is more likely to spread disease than contact with dried faeces, then both gecko species appear to have evolved some ability to distinguish between fresh and dried faecal matter, avoiding the former and ignoring the latter. The unisexual *L. lugubris* is perhaps more sensitive to wet faecal matter than the bisexual *H. frenatus*, because the unisexuals avoided faecal water to a greater extent than bisexuals. Previously Hanley, Volmer & Case (1995) found that populations of *L. lugubris* in the Pacific Basin harboured fewer types of parasites and contained fewer infected individuals than *H. frenatus*. The fact that *L. lugubris* appears to be more sensitive to wet faeces might account for some of the differences in parasitism rates found between the species, although it does not rule out differences in innate immunity between the species.

Geckos of both species were more likely to explore both sides of the enclosure if they first entered the contaminated sphagnum moss. Thus, it does not appear that they are choosing the uncontaminated side of the enclosure via an air borne chemical. This finding supports Schwenk's (1993) hypothesis that geckos have poor vomeronasal senses. It could be that the geckos are using one of their skin sense organs (Matveyeva & Ananjeva, 1995) to make the choice and therefore have to have closer contact with wet faecal matter before avoiding it. Further research needs to be conducted on how the geckos choose an uncontaminated hiding place.

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PHENOTYPIC CORRELATES OF MALE SURVIVORSHIP AND REPRODUCTIVE SUCCESS IN THE STRIPED PLATEAU LIZARD, *SCELOPORUS VIRGATUS*

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Variation in sexual selection among populations and species has been suggested as an explanation for patterns of sexual size dimorphism. The strength of sexual selection on body size and other male traits was studied in a population of *Sceloporus virgatus*, a lizard in which females have a larger average size than males. Sexual selection on male size was found to be weak, with only the very smallest males having a mating disadvantage. This study provides tentative support for a sexual selection-fecundity selection tradeoff as an explanation for sexual size dimorphism in the genus *Sceloporus*, though further studies of the relationship between body size and reproductive success are needed.

INTRODUCTION

The evolution of sexual size dimorphism (SSD) has been the subject of much recent research (Stamps, 1983; Shine, 1994; Fairbairn & Preziosi, 1996; Stamps, Losos & Andrews, 1997). Several hypotheses (not mutually exclusive) have been proposed for the evolution of SSD, including sexual selection favouring large males, fecundity selection favouring large females, and intersexual niche divergence (reviews in Hedrick & Temeles, 1989; Anderson & Vitt, 1990; Madsen & Shine, 1994; Bull & Pamula, 1996; Perry, 1996).

All three hypotheses have been supported by some evidence (Hedrick & Temeles, 1989). The sexual selection hypothesis appears especially promising for lizards, with positive correlations between male body size and some measure of male mating success reported for many species in which males are larger than females (*Anolis garmani*, Trivers, 1976; *Sceloporus jarrovi*, Ruby, 1981; *Anolis carolinensis*, Ruby, 1984; *Uta palmeri*, Hews, 1990; *Ameiva plei*, Censky, 1995; *Crotaphytus collaris*, Baird, Acree & Sloan, 1996). However, studies of lizards and other taxa have shown that the strength of the relationship between male body size and mating success can vary across populations of the same species (the water strider *Gerris odontogaster*, Arnqvist, 1992; Yarrow's spiny lizard *Sceloporus jarrovi*, Dixon, 1993; the soapberry bug *Jadera haematoloma*, Carroll & Salamon, 1995) or among closely related species (*Drosophila*, Markow, Bustoz & Pitnick, 1996). Furthermore, sexual selection for larger male size can occur even in species where females are larger than males (the water strider *Aquarius remigis*, Fairbairn & Preziosi, 1996; the sand lizard *Lacerta agilis*, Olsson & Shine, 1996). The sexual selection hypothesis, then, cannot be a sufficient explanation for SSD in lizards. As in other reptiles (Madsen & Shine, 1994), larger size would be expected to enhance reproductive success of both male and female lizards. Selective pressures might be greater for females in some conditions and for males under other circumstances.

Selective forces on size in both sexes have been examined in species from a variety of taxa (Price, 1984; Howard & Kluge, 1985; Ward, 1988; Harvey, 1990; Olsson & Shine, 1996; Arnqvist, Thornhill & Rowe, 1997). Spiny lizards (*Sceloporus*) present an excellent opportunity to estimate the action of sexual selection and fecundity selection in the evolution of SSD. Species within the genus, and even populations of the same species, vary in the extent and direction of SSD (Fitch, 1978). A positive relationship between female size and clutch size has been documented in many *Sceloporus* species (for example, papers cited in Dunham, Miles & Reznick, 1988). Sexual selection on body size in males has been examined closely only in *S. jarrovi* (Ruby, 1981; Dixon, 1993), a species in which males are larger than females. I studied selection on male size in *S. virgatus*, a species in which females are larger than males. I also considered selection on head dimensions and ventral colouration, as there is evidence that these traits may be associated with male mating success of other lizards (Sigmund, 1983; Hews, 1990; Bull & Pamula, 1996).

METHODS

STUDY POPULATION AND AREA

From 1991 to 1994, I studied a population of the striped plateau lizard (*Sceloporus virgatus*) in the Chiricahua Mountains of south-eastern Arizona, near the Southwestern Research Station of the American Museum of Natural History. Detailed information about this habitat is available in Vinegar (1975). The study area (elevation 1646 m) was situated within North Fork, an ephemeral tributary of Cave Creek. The preferred habitat of *S. virgatus* in this area was small- to medium-sized rocks in and near the creek bed. *S. virgatus* densities dropped sharply as one moved away from the creek; marked individuals were rarely sighted in the grassy woodland on one side of the creek or in the steep rocky area across a road on the other side of the creek. I captured lizards along an approximately 150 m stretch of creek in 1992, and a 300 m length of creek in

1993. The width of the study area varied along the creek according to suitable habitat, but in general the width was 30–60 m.

In both 1992 and 1993, the study area was mapped so that lizard locations could be recorded. I used scan sampling (Altmann, 1974) during daily censuses to record the map location of every lizard I saw. These observations allowed me to determine the home range of each individual. I marked each lizard captured with three small (approximately 0.01 g), coloured seed beads attached at the tail base with plastic monofilament, using a modification of the technique described by Fisher & Muth (1989). This method was usually permanent and allowed for visual identification without having to recapture the animal more often than necessary for morphological measurements. The beads did not appear to interfere with movement or mating. Marked lizards were observed copulating, and some females that had been marked soon after emergence were known to lay fertilised eggs (pers. obs.). The bead marking procedures did not seem to cause infection or tail loss. In other studies of lizards, colour markings did not influence survival (Jones & Ferguson, 1980; Simon & Bissinger, 1983) or mating success (Ruby, 1981). I also clipped three or four toes from each lizard to confirm identity in the rare instance when an individual lost its beads.

I examined selection on male traits in 1992 and 1993, and I considered viability selection in 1992–93 and 1993–94. Age was not known for all individuals in the study, but lizards that were sufficiently small at the time of first capture were known to be in their first spring. Because I started marking animals in 1991, there were known one- and two-year olds in 1992 and known one-, two- and three-year olds in 1993.

MORPHOLOGICAL TRAITS

I measured the body length, body mass, head size, and colour pattern of each lizard caught. I measured snout-vent length (SVL) to the nearest mm with a transparent ruler and body mass to the nearest 0.1 g with Pesola spring scales. I measured head length, head width, and head depth to the nearest 0.1 mm with dial calipers. Head length was the distance from the snout to the posterior margin of the ear opening. Head width was measured as the straight-line distance from ear to ear. Head depth was measured from the top of the head above the eyes to the chin below. I also measured the length and width of the ventral throat patch on the right side with dial calipers, and I counted the number of blue scales in that patch. I calculated the approximate area of the blue throat patch as the area of an ellipse (area = $\frac{1}{2}\pi \times \text{length} \times \text{width}$). I assessed the brightness of blue colouration by matching the colour of the patch against a set of colour standards (Smith, 1975) under field lighting conditions. I converted the blue brightness measurements to numerical scores ranging from 0 (no blue) to 9 (bright blue).

Individual males were captured on different days. For most characters (SVL, mass, head length, head width, head depth, blue throat patch length, and blue throat patch width), I used data from individuals captured more than once to estimate size-specific growth rates. Based on the trait values at first and last capture, I calculated growth rates for each trait for each individual. For each trait, I calculated a regression equation for the relationship between growth rate and body size (SVL). Growth rates of most traits were lower for larger individuals. Blue patch length and width changed little between first and last capture. For each individual, I then calculated the estimated values of all traits on the same day (31 May), using the actual growth rates of the individual (when available) or size-specific growth rates obtained from the regression equations. The number of blue patch scales and the intensity of blue colouration did not differ significantly between the first capture (April or early May) and the second capture (early June) (Abell, 1998), so I used the unmodified values of these traits on the second or only capture.

MALE SURVIVORSHIP AND MATING SUCCESS ESTIMATES

I calculated survivorship using mark-recapture data, and used survivorship from one year to the next as an estimate of male viability. If an individual present in one year was seen in the following year, that lizard was considered a survivor. If the individual was not sighted in the following year, that lizard was assumed to be dead. Most lizards had highly overlapping home ranges from one year to the next. I regularly searched areas adjoining the study site for lizards that had shifted home range. My searches of adjacent areas, along with the fact that the study area was bordered on two sides by less suitable habitat, make it likely that most individuals not seen had died rather than dispersed.

A previous study in the same area found that females that reached sexual maturity in their first spring averaged about 50 mm SVL from 31 May to 14 June (Vinegar, 1975). Reproductive females as small as 46 mm SVL have been collected (Smith, Ballinger & Rose, 1995). For most analyses, I considered a female as potentially sexually mature if her estimated SVL on 31 May was at least 45 mm. The proportion of first-year females that reproduces varies from year to year (Vinegar, 1975; Smith *et al.*, 1995), so it is likely that some of the females between about 45–50 mm SVL did not actually reproduce in the years of the study. I therefore repeated some analyses using only females that were at least 52 mm SVL. Females of this size nearly always reproduce (Smith *et al.*, 1995). I determined the most likely mate or mates of each potentially reproductive female according to the spatial criteria described below. If more than one male was a likely mate, I divided the eggs of that female among the males. Multiple paternity has been documented in clutches of *S. virgatus* (Abell, 1997).

A DNA fingerprinting study in an area adjoining the selection study area suggested that spatial proximity between males and females usually accurately predicts the mate of a particular female (Abell, 1997). Because spatial estimates of paternity were significantly correlated with actual paternity in the sample of lizards used in the fingerprinting study, I used spatial proximity to estimate male mating success.

I used the proximity criterion described in Abell (1997) to estimate mating partners of females. Male-female spatial relationships were divided into three categories on the basis of how often the male and female were sighted within 10 m of each other and the actual distance between the male and female. If a female was observed to have a spatial relationship of higher rank with one male than with any other male, I assumed she mated with that male. If a female had more than one male of equally high spatial rank, I divided her clutch among these males. If a female was never observed within 10 m of a male, I assigned her likely mating partner(s) on the basis of home range overlap alone. In such cases, the clutch was divided among all males with home ranges overlapping that of the female. In the rare cases where a female neither interacted nor overlapped with any male, I assigned that female to the male or males with the closest home range.

MATE FERTILITY ESTIMATES

I estimated mate fertility (Arnold & Duvall, 1994) as well as mating success for all males in my main study population. First, I used longitudinal growth data to estimate the size of all females on a particular day near the end of the breeding season. I then used a regression of clutch size with maternal SVL (Vinegar, 1975) to estimate clutch size of each female. Estimated clutch size was regarded as female fertility. The average mate fertility for a given male was calculated as the average predicted clutch size of his possible mates. When defined this way, the product of a male's mating success (total fractional number of mates) and average mate fertility is equal to the number of eggs likely to be fertilized by that male.

EVALUATION OF SELECTION

The eight morphological measurements included in this analysis were snout-vent length, mass, head length, head width, head depth, blue throat patch area, number of blue scales in patch, and patch colour intensity. I performed principal components analysis (Flury & Riedwyl, 1988) to describe variation among individuals with a smaller number of variables (principal components). Principal components are statistically uncorrelated with each other, so I evaluated the relationship between principal components, and components of fitness by using univariate statistical tests for each principal component.

I performed spatial autocorrelation (Sokal & Oden, 1978a,b) on the principal component scores to test for a

possible association between phenotype and the spatial distribution of males. A lack of significant autocorrelation would suggest that it is reasonable to assume that each lizard was exposed to a similar range of male phenotypes.

Selection on traits can be measured quantitatively with regression techniques, using fitness (survivorship or mating success, for example) as the dependent variable and the character values as independent variables (Lande & Arnold, 1983). Logistic regression (Hosmer & Lemeshow, 1989) is a form of regression suitable for dichotomous outcomes. Survivorship has a dichotomous outcome (live or die), so I performed univariate logistic regression to estimate the strength of viability selection on blue colouration and other characters. Mating success and mate fertility are distinct episodes of selection, so I used established methods for multi-episodic selection (Arnold & Wade, 1984a,b; Wade & Kalisz, 1989; Koenig, Albano & Dickinson, 1991) to estimate the strength of selection on blue colouration and other characters. I used a Fortran program (Koenig *et al.*, 1991) to calculate independent selection gradients.

To examine possible independent effects of age and size, I calculated Kendall's partial correlation coefficient (Siegel & Castellan, 1988) between these variables and each estimate of male reproductive fitness (number of mates and average mate fertility), holding either age or size constant.

RESULTS

MALE MORPHOLOGICAL TRAITS

Including only females that were potentially mature (≥ 45 mm SVL) and observed in the study area for more than a single day, there were 27 females in 1992 and 53 in 1993. Considering only males present for more than one day, there were 33 in 1992 and 57 in 1993, all large

TABLE 1. Principal components analysis, PCI-PCIII for all males, 1993, $n=55$ (14 survived to 1994). * indicates principal component scores higher than 0.7. Results were similar for 1992 ($n=30$).

Character	PCI Body size	PCII Patch size	PCIII Blue colour intensity
Head Depth	0.96*	0.15	0.14
Head Length	0.96*	0.24	0.09
Snout-Vent	0.95*	0.23	0.15
Mass	0.95*	0.25	0.14
Head Width	0.94*	0.24	0.17
Blue Scales	0.14	0.94*	0.15
Blue Area	0.43	0.79*	0.21
Blue Intensity	0.19	0.24	0.95*
Cumulative variance	59.4%	82.2%	95.5%

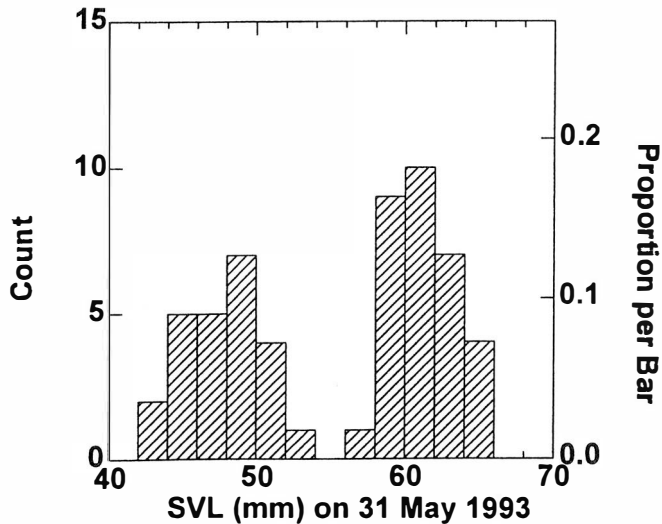


FIG. 1. Frequency distribution of male snout-vent length in 1993.

enough to be sexually mature (Ballinger & Ketels, 1982). Excluding individuals missing some morphological measurements, my sample sizes for the selection analyses were 30 males in 1992 and 55 in 1993.

Males were generally smaller than females (males: mean $\pm$ SE 55.3 ± 1.0 mm in 1993; range 42.7 mm to 65.1 mm in 1993; maximum 65.1 mm from 1991-94; potentially reproductive females: mean $\pm$ SE 58.6 ± 1.4 mm in 1993; range 45.2 mm to 73.9 mm in 1993, maximum 74.3 mm from 1991-94). The distribution of male size was bimodal in both years (Fig. 1). Based on a discontinuity in the size distribution, males at least 56 mm SVL were regarded as large and males less than 54 mm as small. Of the 55 males included in the 1993 selection study, 31 were large and 24 were small. I

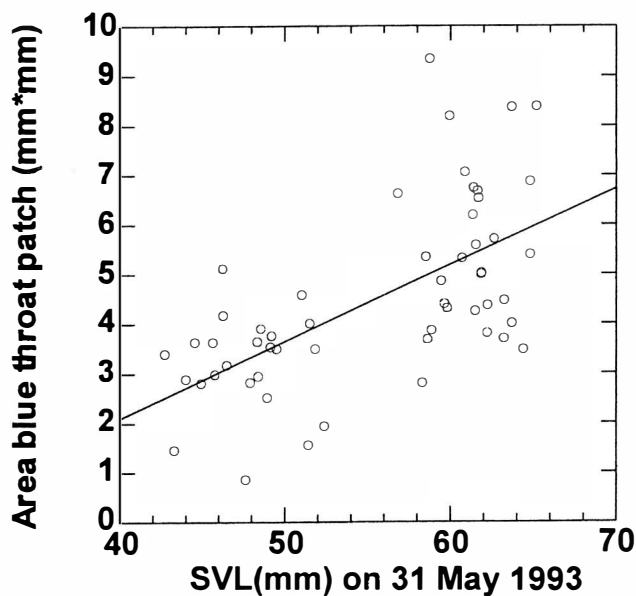


FIG. 2. Area of the blue throat patch relative to snout-vent length in males of *Sceloporus virgatus* in 1993. The regression line is $y = 0.15x - 4.08$, $r^2 = 0.38$, $n = 55$, $P < 0.0001$.

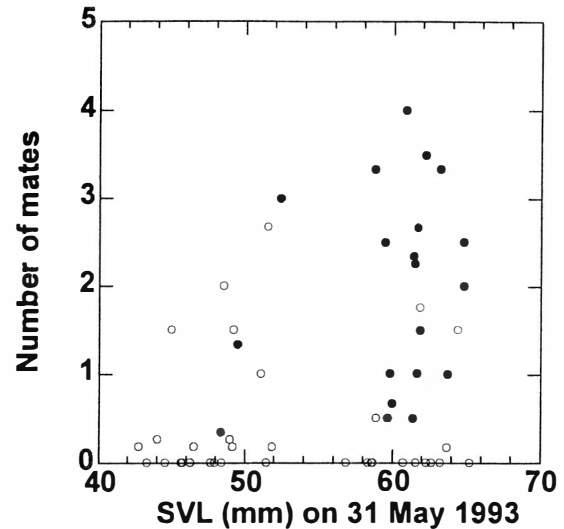


FIG. 3. Male mating success relative to snout-vent length in 1993. Females at least 45 mm SVL were regarded as reproductive. Males with at least one basking partner are shown with closed symbols.

analysed the 1993 data separately for the large and small classes of males. The 1992 data were not analysed separately for two separate size classes because the sample size was smaller and the bimodality less pronounced than in 1993.

Although the blue ventral patch of *S. virgatus* is much smaller than that of most congeners (Smith, 1939), the amount and intensity of blue colouration was highly variable in this population. The brightness of the colour spanned the entire range of the colour standards (cobalt blue dilutions, No. 168 in Smithe, 1975), with a few individuals lighter than the lightest standard. The number of blue scales in a patch ranged from 1 to 19 (mean 8.5, median 8, standard deviation 3.7). The area of the blue throat patch ranged from 0.9 mm² to 9.4 mm² (mean = 4.5 mm²; median = 4.0 mm²; SD = 1.8).

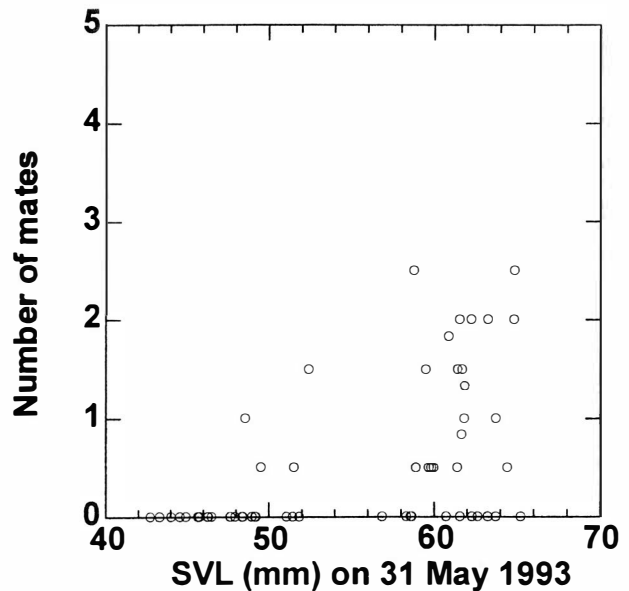


FIG. 4. Male mating success relative to snout-vent length in 1993. Females at least 52 mm SVL were regarded as reproductive.

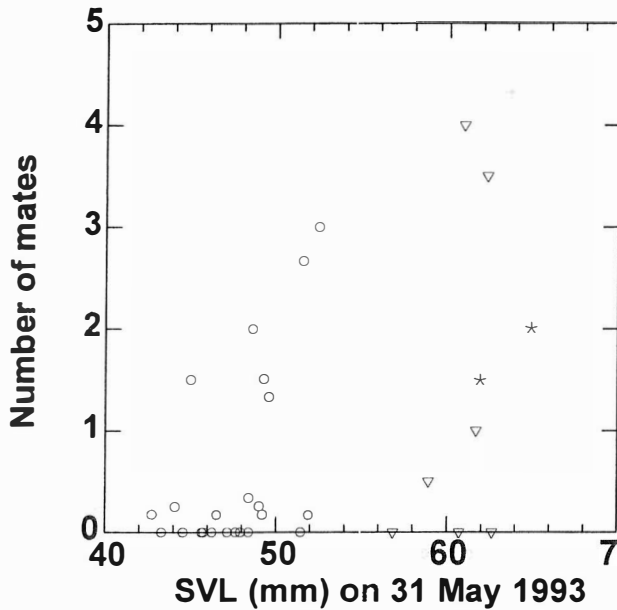


FIG. 5. Mating success relative to snout-vent length for males of known age in 1993. One-year-olds are shown with circles, two-year-olds with triangles, and three-year-olds with asterisks. Females at least 45 mm SVL were regarded as reproductive.

The size of the blue throat patch was significantly correlated with body size (Fig. 2), but only 38% of the variation in patch size could be explained by variation in SVL.

In both 1992 and 1993, three principal components (PCs) accounted for more than 90% of character variance (Table 1). These three PCs were readily interpretable as overall body size, blue patch size and intensity of blue colouration. Similar principal components were found when small and large 1993 males were analysed separately.

No significant autocorrelation was found between male home range overlap and any of the principal components (size PC: Moran statistic $I=0.05$, NS; patch size PC: $I=-0.07$, NS; colour intensity PC: $I=0.04$, NS). That is, there was no tendency for males with overlapping home ranges to be unusually similar or dissimilar in body size, patch size or blue colour intensity.

VIABILITY SELECTION

Of the males for which all morphological data were available, 36.7% (11 out of 30) survived from 1992 to 1993 and 25.5% (14 out of 55) survived from 1993 to 1994. I did not compare survivorship rates between the years statistically because I did not search the study area as frequently in 1994 as in 1993.

For 1992-93, I found no significant association between survivorship (viability) and any principal component. The range of body sizes was similar for males that survived to the following year and for males that did not survive. However, most survivors had a body size PC score greater than 0. For 1993-94, patch size and colour intensity were not significantly associ-

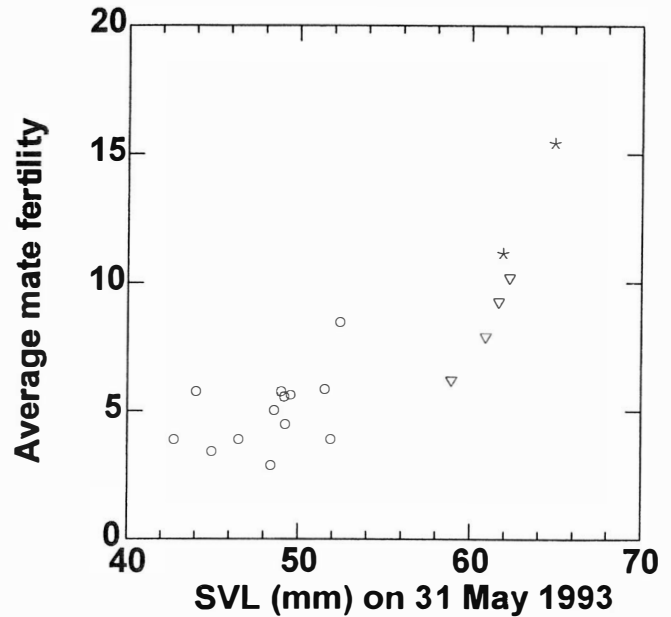


FIG. 6. Average mate fertility relative to snout-vent length for males of known age in 1993. One-year-olds are shown with circles, two-year-olds with triangles, and three-year-olds with asterisks. Only males with at least one mate are included. Females at least 45 mm SVL were regarded as reproductive.

ated with survivorship. Within the group of large males, there was a highly significant tendency for the largest males to have higher survivorship (logistic regression coefficient = 1.33 ± 0.45 SE, $G=6.889$, $P < 0.01$; 10 of 31 large males survived to 1994). No relationship between size and survivorship was found in the group of small males (4 of 24 small males survived to 1994).

SEXUAL SELECTION AND MATE FERTILITY SELECTION

There were no significant or nearly significant sexual selection or mate fertility selection gradients on the size of the blue patch or the intensity of the colouration. Thus, there was no evidence for directional selection on blue colouration in *S. virgatus*, in either year or within either size class.

The estimates of mating success had a skewed distribution, with most males having low success and a few males having much greater success. When all males were considered together, larger body size was significantly associated with greater mating success in both years (1992: $\beta' = 0.45 \pm 0.16$ SE; $0.005 < P < 0.01$; 1993: $\beta' = 0.37 \pm 0.13$ SE; $0.005 < P < 0.01$), but there was no significant association between male size and mate fertility. A scatterplot of the 1993 data suggested a size beyond which greater size was not associated with enhanced mating success (Fig. 3). When large and small males were considered separately, no significant sexual selection or mate fertility gradients were found on the body size principal component calculated from large males alone. In the data set of small males, there was a highly significant tendency for larger males to have more mates ($\beta' = 0.73 \pm 0.26$ SE; $0.005 < P < 0.01$), but

TABLE 2. Kendall's partial correlation coefficient (T) between male reproductive fitness (mating success or mate fertility) and SVL (controlling for age) or age (controlling for SVL). All potentially reproductive females (45 mm SVL) were included. * $P < 0.05$.

		SVL (controlling for age)	Age (controlling for size)
Fitness component	<i>n</i>	<i>T</i>	<i>T</i>
Mating success			
1992	19	0.0871	0.0700
1993	33	0.2545*	0.0208
Mate fertility			
1992	13	-0.0184	0.3521
1993	19	0.3927*	0.4028*

there was no significant relationship between size and mate fertility.

I repeated the 1993 selection analysis using only females that were almost certainly reproductive (≥ 52 mm SVL on 31 May). Results were similar to those described above based on all potentially reproductive females (≥ 45 mm SVL). There was no significant sexual selection or mate fertility selection on blue colouration. There was also no significant relationship between male body size and mate fertility. When all males were considered together, there was very strong sexual selection for larger males (Fig. 4; $\beta' = 0.64 \pm 0.15$ SE, $P < 0.001$). Within the group of large males, there was no significant selection on male body size. Within the group of small males, larger males had higher mating success ($\beta' = 1.20 \pm 0.51$ SE, $0.01 < P < 0.02$). The yearling females (approximately 45–52 mm SVL) had been assigned as mates of males of all sizes, so the selection results were essentially the same whether these females were included or not.

When the effects of age were controlled for with partial correlation analysis, body size was significantly positively correlated with male mating success and mate fertility in 1993 (Figs. 5, 6), but not in 1992 (Table 2). When the effects of size were controlled for with partial correlation analysis, age was not significantly correlated with male mating success in either 1992 or 1993 (Table 2, Fig. 5). In 1993 there was a significant positive correlation of age with average mate fertility (Fig. 6), and there was a corresponding non-significant trend in 1992 (Table 2).

DISCUSSION

Larger males tended to have more mates, but this does not necessarily imply continual directional selection for indefinitely larger males. Only the very smallest males were at a mating disadvantage. This relationship between male size and mating success is similar to that reported for *Crotaphytus collaris* (Baird *et al.*, 1996).

Traits other than the morphological characters considered in this study are probably most important in determining male fitness. The closest category of male-female spatial proximity was termed a basking partnership (Abell, 1997). Males that maintained at least one basking partnership usually had relatively high values of mating success (Fig. 2) and average mate fertility. The relationship between basking partnerships and mating success was not due simply to the fact that males in the analysis were assigned as mates of their female basking partners. Males with basking partners apparently were successful at obtaining additional mates.

Larger (and hence older) males were most successful at maintaining basking partnerships (Fig. 3; Abell, 1997). Small males were seldom observed to engage in such relationships. Males sometimes maintained basking partnerships in more than one year, but I do not have enough data to estimate the frequency of long-term partnerships. Of the five males known to have basking partnerships in 1992, three survived to 1993. One of them re-established a basking partnership with the same female. The partners of the other two males did not survive to 1993. One of these males established basking partnerships with two different females in 1993 and the other male was apparently a roving, non-territorial individual in 1993. These data indicate high variability in male-female interactions within and between years.

Age was not associated with the number of mates in either year. This result is similar to that of Olsson & Shine (1996) for the sand lizard *Lacerta agilis*, also a species in which females average larger than males. However, older males did mate with larger, and hence more fertile, females. Mate choice experiments by Fitzgerald (1982) showed that males of *S. virgatus* choose the larger female when presented with two fertile females as potential mates.

The relationship between female size and clutch size is stronger in *S. virgatus* than in most other *Sceloporus* species (Abell, unpubl. obs.). For example, in four studies of *S. jarrovi* the slope of the regression line between female SVL and litter size ranged from 0.24 to 0.31, whereas in five studies of *S. virgatus* the slope of the regression line between female SVL and clutch size ranged from 0.27 to 0.64 ($P < 0.05$ for all regressions; *S. jarrovi*: slope 0.28 in Goldberg, 1971; 0.24 in Tinkle & Hadley, 1973; 0.31 and 0.29 in Ballinger, 1979; 0.26 in Ruby & Dunham, 1984; *S. virgatus*: 0.64 for data extracted from Cole, 1963; 0.44, 0.46 and 0.53 in Vinegar, 1975; 0.64, 0.43 and 0.53 for data extracted from Smith, 1977; 0.40 in Smith *et al.*, 1995; 0.27 and 0.59 in Abell, in press). The sharp increase in clutch size for a relatively small increase in maternal size suggests strong fecundity selection for large females in *S. virgatus*. The present study shows that sexual selection on male size is weak in *S. virgatus*, a species with females larger than males. In contrast, male reproductive success was closely associated with body size in one study of *S. jarrovi*, a species with males larger than fe-

males (Ruby, 1981). Another study of *S. jarrovi* found significant sexual selection for larger males in the higher elevation, higher density population but not in the lower elevation, lower density population (Dixon, 1993). These results provide tentative support for a sexual selection-fecundity selection trade-off as an explanation of sexual size dimorphism in *Sceloporus* lizards. Additional studies of the relationship between body size and fitness are needed for a rigorous comparative analysis.

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PITS VERSUS PATTERNS: EFFECTS OF TRANSPONDERS ON RECAPTURE RATE AND BODY CONDITION OF DANUBE CRESTED NEWTS (*TRITURUS DOBROGICUS*) AND COMMON SPADEFOOT TOADS (*PELOBATES FUSCUS*)

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During a long-term study (1987-1996) near Vienna (Austria), individual Danube crested newts (*Triturus dobrogicus*) and common spadefoot toads (*Pelobates fuscus*) were registered by photographs of highly variable skin patterns, and the implantation of Passive Integrated Transponders (PIT tags). To test for potential detrimental effects of the electronic tags, we compared within-year (for *P. fuscus*) and between-year (for *T. dobrogicus*) recapture rates and body condition index (CI) of individuals marked with the two alternative techniques. No significant negative effects of PIT tags on CI and recapture rate could be demonstrated for either species. There was a decrease in body condition and recapture rates in the study years 1995 and 1996, but can be attributed to population trends rather than to the different marking methods. For recaptured *T. dobrogicus* a 10.5% loss of PIT tags was observed.

INTRODUCTION

One of the major difficulties in conducting studies on free-ranging amphibian populations is the selection of a suitable marking technique. Compared to other vertebrates, amphibians are especially difficult to mark permanently on an individual level (for reviews see Ferner, 1979; Donnelly, Guyer, Juterbock & Alford, 1994; Henle, Kuhn, Podlousky, Schmid-Loske & Bender, 1997). Toe clipping is the most commonly applied technique, but may pose some problems: the number of individual codes is limited, and for European newts, this technique is applicable only for a limited time scale due to their regeneration ability (Andreone, 1986). In some cases, a negative effect of toe clipping on recapture rates was reported (Clarke, 1972; Golay & Durrer, 1994). Being a putatively non-invasive method, pictures of individual spot patterns have been used successfully for identification of appropriate species (Hagström, 1973; Gill, 1978; Arntzen & Teunis, 1993; Jehle, Hödl & Thonke, 1995). In recent years, the implantation of Passive Integrated Transponders (PIT tags) was introduced as a marking method for reptiles and amphibians (Camper & Dixon, 1988). PIT tags are glass-encased electromagnetic coils (approx. size: 10 x 2.1 mm) bearing a unique alphanumeric code that is read by generating a low-frequency electromagnetic signal with an external reader. Several field studies have been conducted to test for potential harmful effects of implanted transponders on snakes and lizards (Germano & Williams, 1993; Keck, 1994; Jemison, Bishop, May & Farrell, 1995). For amphibians, the application of this technique has been thoroughly described (Sinsch, 1992; Faber, 1997), and some laboratory and field-enclosure studies exist comparing PIT-tagged with untagged toads and newts over periods of up to a few months (Corn, 1992; Fasola, Barbieri & Canova, 1993). So far, only a single investigation on free-ranging amphibian populations has been

conducted to test for detrimental effects of implanted transponders by calibrating the results with alternative techniques [Brown (1997), on *Bufo bufo* and *Rana temporaria*]. For most species on which this method has already been applied, long-term field data are lacking.

During a long-term study near Vienna (Austria), two out of eleven investigated species (*Pelobates fuscus*, the common spadefoot toad, and *Triturus dobrogicus*, the Danube crested newt) were recognizable individually (Thonke, Jehle & Hödl, 1994; Jehle *et al.*, 1995; Hödl, Jehle & Gollmann 1997). Individuals were registered using photographs of ventral spot patterns over six study years (for *T. dobrogicus*), and using dorsal patterns over seven years (for *P. fuscus*). As the workload for comparing pictures increased with study duration, the method of pattern mapping was replaced by the implantation of PIT tags in the 8th year (Hödl *et al.*, 1997). This enabled us to test possible detrimental effects of implanted transponders on these two species. In this paper we present a comparison of within-year and year-to-year recapture rates and body condition of two amphibian species in the field, emphasizing differences between individuals marked with the two alternative techniques. Although Faber (1997) presents field results on recapture rates of PIT-tagged alpine newts (*T. alpestris*), data derived from free-ranging urodeles are for the first time compared with those from individuals recognizable by other methods.

MATERIALS AND METHODS

The study was conducted from 1986 to 1997 (with the exception of 1988). Data presented here encompass the years 1987 and 1989-1995 for the spadefoot toad; and 1987 and 1989-1996 for the crested newt. Study animals were collected using a drift fence and pitfall traps completely encircling the breeding pond (Dodd & Scott, 1994; Arntzen, Oldham & Latham, 1995). A de-

tailed description of the study site and the fence system is given in Hödl *et al.* (1997). The traps were checked daily between 0700 and 0900 hrs, and the animals were processed immediately after capture. For measuring, weighing, photography and PIT tag implantation, individuals were transported to a laboratory about 1 km away from the study pond. As amphibians can substantially lose mass due to water loss in a dry environment (pers. obs.), all study animals were immersed in water for at least 10 min and then weighed to the nearest 0.1 g using an electronic balance. Snout-vent length (SVL) was measured with a vernier calliper to the nearest 1 mm (1987, 1989) or 0.1 mm (from 1990 onwards). In *T. dobrogicus* this measurement was taken from the tip of the head to the end of the cloaca, while the same in *P. fuscus* was from the tip of the head to the posterior end of the body. For *T. dobrogicus*, several attempts to straighten the wriggling animal were necessary before taking the measurement. After processing, the study animals were released on the opposite side of the drift fence to the point of capture, the presumed direction of migration. Transponders were applied from August 1994 onwards; prior to this date individuals were identified by photography only. Due to their small size, *T. dobrogicus* weighing less than 2 g (after Fasola *et al.*, 1992) and *P. fuscus* metamorphs were photographed but not PIT tagged. Once a PIT-tagged individual was recaptured, no further photographs were taken. In order to test whether an individual had lost its tag, adult catches without transponders were both PIT tagged and photographed, and the obtained pictures were compared with previous photographs.

Prior to implantation, the study specimens were anaesthetized using a 1:500-1:1000 solution of MS 222 (Ethyl-m-Aminobenzoate-Methanosulfonate, Sandoz). After 10-20 minutes, the muscular system was relaxed and the animals stopped moving. For *T. dobrogicus*, the transponders [Trovan (Germany), supplied by Datatronic (Wr. Neustadt, Austria)] were implanted in the abdominal body cavity using a sterile hypodermal syringe supplied by the vendor. The needle was inserted laterally in the posterior one-third of the body, oriented with its beveled surface away from the venter and directed towards the anterior part of the body. To prevent injury to the intestines, the piece of skin where the needle was positioned was lifted with two fingers, enabling an insertion almost parallel to the anterior-posterior body axis. Visible bleeding occurred only in rare cases. After the implantation, the wound was closed using the tissue glue VET SEAL (after Faber, 1997). For *P. fuscus*, PIT tags were injected into the subcutaneous lateral lymph sacs. Basically, the same technique was used but without the need to penetrate the body cavity. No obvious complications occurred during the implantation procedure. The animals were released as soon as they had recovered from anaesthesia.

Amphibians entering a breeding pond are in a different physiological condition than animals leaving a

reproductive site (Verrell, Halliday & Griffiths, 1986). Therefore, apart from separating the sexes, immigrants and emigrants were treated differently during the statistical analysis. For female *P. fuscus*, we observed a substantial mass loss during the aquatic phase, most probably due to oviposition (unpublished data). *Pelobates fuscus* stay for a relatively short time inside the fenced area [average 1987, 1989-1995: 22.9 days (females), 37.3 days (males), data from Wiener, 1997]. They were PIT tagged for the first time when entering the pond in 1995 and re-registered when leaving the breeding site in the same season. Therefore, within-year recapture rates were compared for this species.

In 1994, 35 (48%) of the registered *T. dobrogicus* (13% of the total population including juveniles) were captured after August and PIT tagged. A comparison of between-year recapture rates between 1994/95 and other study years is therefore possible, although it has to be considered that individuals from 1994 were only PIT tagged rather late in that year, whereas recaptures in 1995 were PIT tagged over the whole season.

Chi-square tests with Yates' correction were used, for samples with both categories exceeding zero, to test for deviations from even distributions of recapture rates of pattern-mapped individuals and individuals with transponders. A condition index (CI) was calculated for both species with the formula $CI = (\text{mass [g]})/(\text{SVL [cm]})^3 \times 100$ (Weatherley, 1972). The Mann-Whitney *U*-test was used to test for significant differences in CI in the same year; for significant differences between study years, analysis of variance (ANOVA) was applied.

RESULTS

Adult individuals without transponders, which were captured after the introduction of PIT tags, were both photographed and PIT-tagged, and they were compared with the photographs of the captures from previous

TABLE 1. Between-year recapture rates for *T. dobrogicus*. Data from the first five rows were obtained using pattern mapping. See text for juveniles and subadults. * Data from Jehle *et al.* (1995) and Ellinger & Jehle (1997).

<i>Triturus dobrogicus</i>	Females	Males
1989/90*	15/44 (34%)	20/43 (47%)
1990/91*	11/32 (34%)	19/36 (53%)
1991/92*	18/45 (40%)	15/44 (34%)
1992/93*	18/40 (45%)	9/41 (22%)
1993/94*	10/41 (24%)	7/32 (22%)
Weighted mean 1989/90-93/94	36%	36%
1994/95		
PIT-tagged	7/25 (28%)	4/10 (40%)
Pattern-mapped	8/36 (22%)	6/23 (26%)
χ^2	$P > 0.05$	$P > 0.05$
1995/96		
PIT-tagged	5/39 (13%)	0/20 (0%)
Pattern-mapped	-	-

TABLE 2. Within-year recapture rates of *P. fuscus*. Data without special indications refer to pattern-mapped individuals.

	Females	Males
1989	60/78 (77%)	77/138 (57%)
1990	31/58 (53%)	34/55 (62%)
1991	19/30 (63%)	15/27 (56%)
1992	22/32 (69%)	28/48 (58%)
1993	48/66 (73%)	101/137 (74%)
1994	117/146 (80%)	95/155 (61%)
Weighted mean 1989-1994	72%	62%
1995 (PIT-tagged)	67/111 (60%)	106/139 (76%)

years. With this procedure, two lost transponders (0.8% of the recaptured individuals) were recorded for *P. fuscus*, and six for *T. dobrogicus* (10.5% of all within and between-year recaptures).

In 1994/95, PIT-tagged male and female *T. dobrogicus* had higher (but not significantly higher) year-to-year recapture rates than individuals which were only photographed (Table 1). Adult recapture rates between 1995 and 1996 were very low. Between 1994 and 1995, none of the 40 juveniles with transponders was recaptured, whereas 7% ($n_{\text{total}} = 645$) non-PIT-tagged *T. dobrogicus* were recaptured. For juveniles from 1995, 18% ($n_{\text{total}} = 11$) of PIT-tagged individuals and 9% ($n_{\text{total}} = 877$) of pattern-mapped indi-

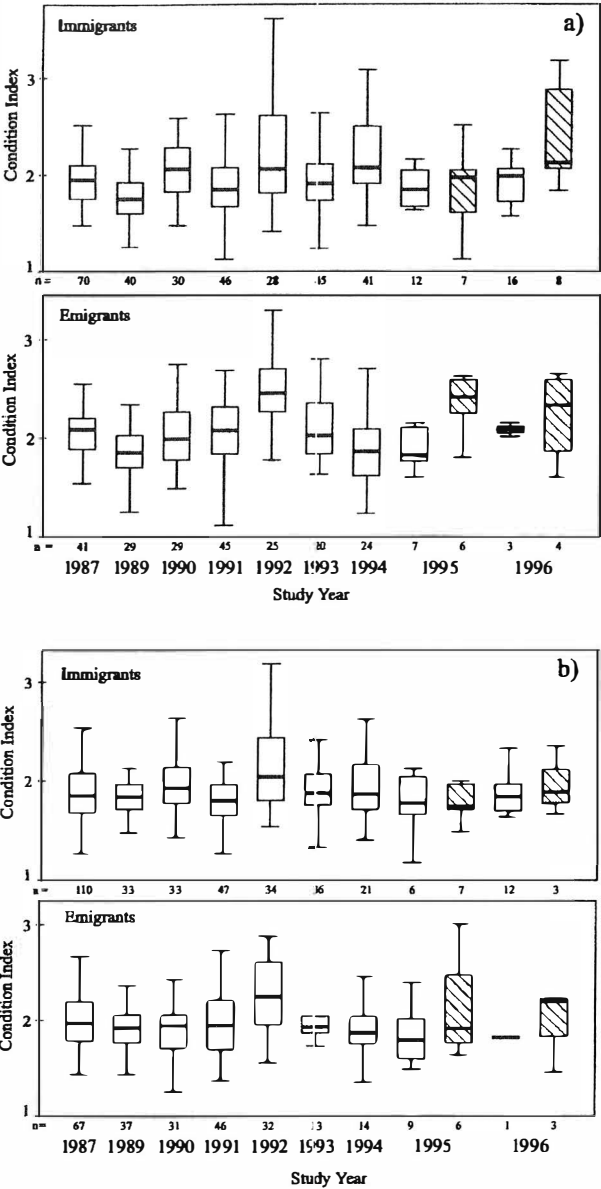


FIG. 1. Condition index of *T. dobrogicus*. Boxplots without special indications represent data obtained from pattern-mapped individuals, hatched boxplots represent data obtained from PIT-tagged individuals. (a) Females, (b) Males.

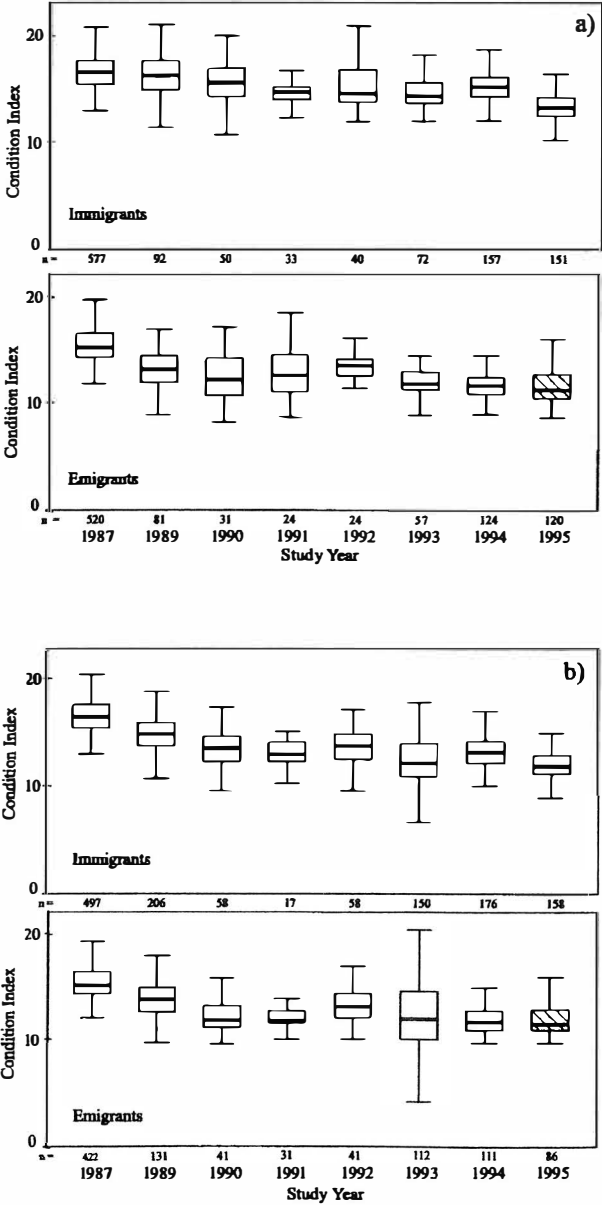


FIG. 2. Condition index of *P. fuscus*. Boxplots without special indications represent data obtained from pattern-mapped individuals, hatched boxplots (emigrants from 1995) represent data obtained from PIT-tagged individuals. (a) Females, (b) Males.

viduals were recaptured at the pond in 1996. Recapture rates for PIT-tagged female *P. fuscus* were low, but within the range of values obtained from previous years (Table 2). PIT-tagged male *P. fuscus* showed the highest value recorded.

When photographs of skin patterns were used exclusively for both species, CI values differed significantly between the study years (ANOVA: *F*-values ranging from 3.48 for *T. dobrogicus* male emigrants to 108.84 for *P. fuscus* female emigrants, $P < 0.01$ in all cases). Therefore, statistical analysis between study years to test the effect of the identification method is not appropriate. In general, *T. dobrogicus* had a higher condition index when leaving the pond (Fig. 1), whereas *P. fuscus* had higher values when entering the breeding site (Fig. 2). For both species, the study years 1995 and 1996 were characterized by low CI values compared to previous years. In seven out of eight cases, PIT-tagged *T. dobrogicus* had higher CI values than pattern mapped individuals (Fig. 1); in one case this difference is actually significant (female emigrants of 1995, $P < 0.05$). PIT-tagged *P. fuscus* showed the lowest condition index recorded (Fig. 2). However, equally low CI values of the non-PIT-tagged immigrants of the same year were observed, suggesting a year-specific rather than a methodological effect for both sexes.

DISCUSSION

For both scientific and conservation purposes, marking techniques should have no negative influence on the animals under investigation. No significant long-term detrimental effects of transponders on recapture rate and body condition could be demonstrated in this study. However, the influence of habitat-related factors and/or climatic conditions on yearly variation in recapture rates and especially CI values seemed to be substantial, making statistical tests between study years impossible. Nevertheless, CI was used for analysis of somatic condition. The observed values of individuals marked with transponders could be calibrated against variability of CI in general, and, in the case of *P. fuscus*, PIT-tagged emigrants could be compared to non-PIT-tagged immigrants in 1995. Individual growth rates were very variable in different seasons, and were therefore less useful as the animals were captured only at irregular intervals. The data presented in Brown (1997) on *B. bufo* and *R. temporaria*, showing no differences in mass-length relationships and recapture rates between PIT-tagged and dye-marked individuals, are statistically more meaningful, as both methods were applied simultaneously. However, Brown (1997) had sufficient data only for male *B. bufo*, whereas we present a data set for two species and both sexes, divided into immigrants and emigrants.

This study depends upon the assumption that pattern mapping has no effect on the animal and is error-free. Error levels of the photographic method were not included in the analysis. Several field workers independently checked the photographs, and the pro-

portion of individuals which were registered twice at the same side of the fence (i.e. erroneously matched captures or individuals which trespassed the fence without registration) was as low as 3.51% (Hödl *et al.* 1997). Thus, the error level for pattern-matching is assumed to be low (<3%), although in another study, a value of 14% was recorded (cited in Sweeney, Oldham, Brown & Jones, 1995).

The *T. dobrogicus* recapture rate between 1995 and 1996 was only 9% compared to an average of 41% between 1989 and 1994. Only one newt, PIT tagged in 1994, was re-registered in 1996. The decrease of CI values in 1995 and 1996 for both species is consistent with a reduction of the adult population size (especially for *T. dobrogicus*, unpublished data). However, the non-PIT tagged juvenile *T. dobrogicus* emerging from the pond in 1996 showed a 30.0% lower mass and a 19.8% lower total length compared to the mean of 1987 and 1989-1995. Recapture rates of pattern-mapped juveniles from 1995/96 were reduced to 57% of the mean value from 1989/90 - 1993/94. Non-PIT tagged emigrating toads from 1995 also had very low CI values (Fig. 2). Thus, year-specific population traits seem to be responsible for the observed difference in the measured parameters rather than effects of transponders.

Higher recapture rates of PIT-tagged newts in 1994/95 (Table 1) can be attributed to the fact that they were exclusively registered in the second half of 1994, whereas pattern-mapped individuals were last seen when entering the pond in spring. Therefore, they were traced back over a longer time span, and were exposed to the risk of mortality for longer. The fact that PIT-tagged *T. dobrogicus* had (in one case, significantly) higher CI values when recaptured in 1995 may be due to the fact that most of the pattern-mapped individuals (i.e. individuals which were registered before, but not after the breeding season of 1994, and again in 1995) spent the winter inside the fenced area or even in the pond. Only two adults were PIT tagged in the autumn when entering the pond. Thus, the body condition mainly seems to reflect this difference in their life history.

No loss of transponders has been reported previously, neither for studies on captive amphibians (Fasola *et al.*, 1993; Brown, 1997), nor for field studies (Faber, 1997). To detect transponder losses in the field, animals additionally need to be registered with an alternative method when being PIT tagged. In our investigations, one tenth of *T. dobrogicus* but less than 1% of *P. fuscus* lost their tag. One possible explanation for this bias is that the healing of an opening in the newt's body cavity takes more time than a wound of the rather thin anuran skin, and that the tissue glue was shed during ecdysis, resulting in an opening through which the transponder could exit during the first few days after implantation. One individual was found in the field with an open wound (Tamnig, pers. comm.). A 10.5% loss is a rather high rate, even when recapture rates do not seem to be affected. A closing of the wound with sutures does not

seem to be appropriate, as the incision is only approx. 2 mm in diameter. However, we suggest that this problem can be overcome by more thoroughly closing the body opening with tissue glue, and by inserting the PIT tag more deeply in the body cavity (at least 5 mm away from the opening) before closing the wound.

In sum, the variation of recapture rate and condition index is mainly caused by differences in population traits. However, it is very important to state that being unable to prove significant harmful effects is completely different from proving that a marking technique definitely is not detrimental. For example, although six *T. dobrogicus* were re-registered after having lost their transponder, deaths of a few individuals caused by an opened body cavity cannot be completely excluded. Juveniles, the life stage on which currently the most urgent need for studies exists, can be identified with pictures, but as a rule are too small for transponders and microtags may be the only option (e.g. Sinsch 1997a,b). Although recommendations are very difficult, our data provide evidence that PIT tags, when applied very carefully, can be used successfully for field studies on adult *T. dobrogicus*, *P. fuscus*, and on closely related species of similar size.

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DISTRIBUTION AND HABITATS OF SCHREIBER'S GREEN LIZARD (*LACERTA SCHREIBERI*) IN PORTUGAL

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We describe the habitats of the endemic Iberian lizard *Lacerta schreiberi* in Portugal and determine those habitat components that best explain the presence of the species. The geographic distribution of *L. schreiberi* in Portugal was also determined through extensive field surveys. The previously known distribution area was enlarged by 150% and new isolated populations were detected. The area occupied by the three previously known southern isolates was delimited and increased by 300%. The species is usually restricted to the margins of rivers and streams. Major habitat characteristics that correlate with the presence of the species are water velocity and quality, dominant species in the tree and shrub strata, streams surrounding the biotopes, and altitude. Nevertheless, the selection patterns that *L. schreiberi* seems to exhibit are only a consequence of its preference for the Atlantic climate. Consequently the presence of the species in a given watercourse seems to be more dependent on the climate of that region than on the intrinsic characteristics of that watercourse.

INTRODUCTION

Habitat destruction and fragmentation have been considered the major factors of species decline and extinction (e.g. Dickman, 1987; Soulé, Alberts & Bolger, 1992). Therefore identification and characterization of species habitats is a fundamental step in any conservation plan. Several methods for evaluating habitats (US Fish and Wildlife Service, 1980) and their suitability (US Fish and Wildlife Service, 1981; Wakeley, 1988) have been developed, as well as ways of integrating them with other ecological information (Wildlife Working Group, 1991).

Schreiber's Green Lizard (*Lacerta schreiberi*, Bedriaga 1878) has been studied in Portugal in order to establish a conservation strategy, in which we plan to incorporate habitat information (Brito *et al.*, submitted a). Our aim in this paper is to identify precisely *L. schreiberi* habitats (since it is already known that it inhabits margins of watercourses, e.g. Barbadillo, 1987) and to detect which habitat components correlate most with the presence or absence of the species. Simultaneously we describe the distribution of the species, with detailed comments for the isolated populations, since a previous distribution map has been presented in Brito, Brito-e-Abreu, Paulo, Rosa & Crespo (1996), but on a more general scale.

L. schreiberi is a medium-sized lizard (snout-vent length 117-120 mm) endemic to the Iberian Peninsula, with a markedly Atlantic distribution. In Spain it occurs in Cantábria, Astúrias, Galicia and the Central System mountains, and in Portugal it is present north of the river Tejo. In both countries there are several known isolated populations in the South (Marco & Pollo, 1993; Brito *et al.*, 1996), such as the Sintra, Monchique/Cercal and S. Mamede mountains in Portugal, and the Las Villuercas, Toledo, San Andrés and Morena mountains in Spain. These isolated populations occur in mountains which are "Atlantic islands" surrounded by

areas of strong Mediterranean influence. *L. schreiberi* inhabits the margins of streams and rivers, generally in very humid places, being strongly influenced by climatic variables, especially rain and insolation (Brito *et al.*, 1996; submitted b). It is a good climber of stone walls and bushes, and feeds mostly on Coleoptera, Formicidae and Diptera (Marco & Pérez-Mellado, 1988).

METHODOLOGY

DISTRIBUTION

Selection of the sampling area was based on the previously known distribution, assembled from the works of Malkmus (1981; 1995), Barbadillo (1987), De La Riva (1987), Crespo & Oliveira (1989) and Marco & Pollo (1993). Fieldwork was carried out between March and August in the years 1994 to 1996. We sampled 781 watercourses and margins, distributed in 412 UTM 10 x 10 km squares (Fig. 1), which represents 44% of the area of Portugal. We only sampled watercourses and adjacent areas because this is the habitat to which the species is mainly restricted (Malkmus, 1981, 1995; Barbadillo, 1987; De la Riva, 1987; Crespo & Oliveira, 1989). The field sampling took the form of a visual encounter survey lasting about 20 min and/or 100 m of streamside habitat.

All information was compiled in a database and represented as occurrence points in UTM 10 x 10 km squares. For the isolated populations we used a more precise scale - UTM 1 x 1 km. For Sintra mountain we also used information collected by Marques, Paulo & Crespo (1995), while establishing a conservation plan for the herpetofauna of that area.

HABITAT

In every sampled watercourse we also gathered information on variables that characterized the habitat. In Table 1 we present the thirteen variables used and lev-

TABLE 1. Variables and levels used for characterization of the watercourses.

Variable	Levels
Altitude	Metres above sea level
Orientation	N, W (includes NW and SW), S and E (includes NE and SE)
Average width	0.0-1.0, 1.0-2.5, 2.5-5.0, 5.0-7.5 and >7.5 m
Depth	0.00-0.25, 0.25-0.50, 0.50-0.75, 0.75-1.00, >1.00 m
Water velocity	Zero, medium, fast and very fast
Water quality	Dry, clear, inorganic turbidity, organic turbidity, polluted and very polluted
Margin substratum	Sand, soil, stone, soil and stone, rock piles, stone walls and others (clay and cement)
Dominant species in tree strata	Narrow-leaf ash (<i>Fraxinus angustifolia</i>), pine (<i>Pinus pinaster</i>), Mediterranean trees (<i>Quercus faginea</i> , <i>Q. rotundifolia</i> , <i>Q. suber</i> , <i>Ceratonia siliqua</i> and <i>Nerium oleander</i>), poplars (<i>Populus</i> sp.), exotic species (<i>Eucalyptus</i> sp., <i>Cupressus</i> sp. and <i>Acacia</i> sp.), orchards, marsh species (<i>Arundo donax</i> and <i>Phragmites</i> sp.), willows (<i>Salix</i> sp.) and Atlantic trees (<i>Alnus glutinosa</i> , <i>Betula pubescens</i> , <i>Castanea sativa</i> , <i>Quercus robur</i> and <i>Q. pyrenaica</i>).
Dominant species in shrub strata	Mediterranean species (<i>Quercus coccifera</i> , <i>Pistacia lentiscus</i> , <i>Tamarix africana</i> and some plants from families Cistaceae and Ericaceae), brambles (<i>Rubus</i> sp.), marsh species (<i>Juncus</i> sp., <i>Phragmites</i> sp. and <i>Scirpus</i> sp.), Atlantic species (<i>Alnus glutinosa</i> and some plants from families Cistaceae and Ericaceae), gramineous (unidentified plants) and other species (exotics, <i>Crataegus monogyna</i> , <i>Smilax aspersa</i> and <i>Cytisus</i> sp.)
Percent cover of tree + shrub stratum	0-20, 20-40, 40-60, 60-80 and 80-100%
Surrounding biotope	Olive cultures, shrubby areas, pinewoods (<i>Pinus pinaster</i>), diverse pinewoods (<i>P. pinaster</i> with dense undergrowth, or other less represented species), cork and holm oak groves (<i>Q. suber</i> and <i>Q. rotundifolia</i>) groves, <i>Eucalyptus</i> sp. forests, urban areas (villages or towns), irrigated and arid agricultural systems, English (<i>Quercus robur</i>) and Pyrenean (<i>Q. pyrenaica</i>) oak forests, and distinct montane agricultural valleys with and without pine woods.
Intensity of human activities	No human activities or abandoned agricultural areas, leisure areas, agricultural areas with few houses, orchards, vineyards, apiculture or pastures; previous level with tarred roads or more houses; small villages; towns, cities or any other human activities, that completely destroy the surrounding area.

els considered. Altitude was only used as a variable for the isolated populations because in other works at a national scale (Brito *et al.*, 1996; submitted b) it does not explain the presence of the species. The variables water velocity, water quality and percentage of cover of each stratum were locally estimated through visual evaluation.

According to Brito *et al.* (1996; submitted b), climate, particularly precipitation and insolation, is strongly correlated with the presence of *L. schreiberi*. Therefore it is difficult to separate habitat selection from presence/absence due to climate constraints. In the northern region climate is relatively constant and suitable for the presence of *L. schreiberi*, while the isolated populations appear in less favourable climatic regions (Brito *et al.*, 1996; submitted b). Therefore we analysed separately data from the northern region and from the isolated populations.

For the three regions considered - North of the river Tejo, Monchique/Cercal and S. Mamede - the statistical treatment was identical. In all variables we used Chi-squared tests (Seigel & Castellan, 1988) to test if the relation between the different levels of variables and the presence or absence of the species is significant or if it is only due to different sampling efforts. For each class of a given variable, the difference between observed and expected frequencies of presence of the species indicates the preference or avoidance for that class.

RESULTS

DISTRIBUTION

Fig. 2 shows the distribution of *L. schreiberi* in Portugal, with an increase of 150% relative to the previously known range. Of the total area of the Portuguese distribution, 61% of the squares correspond to

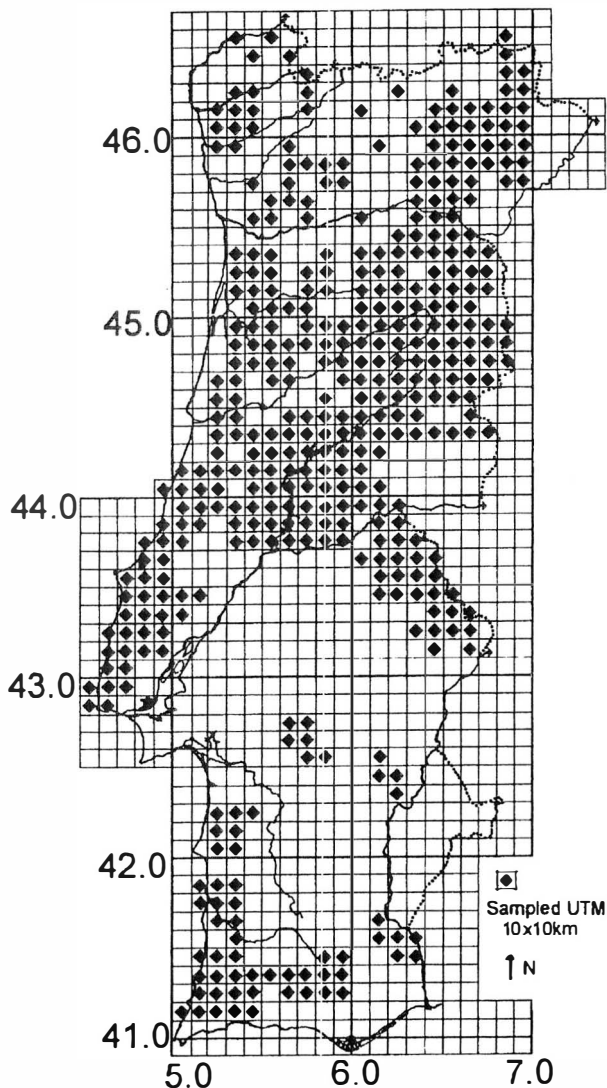
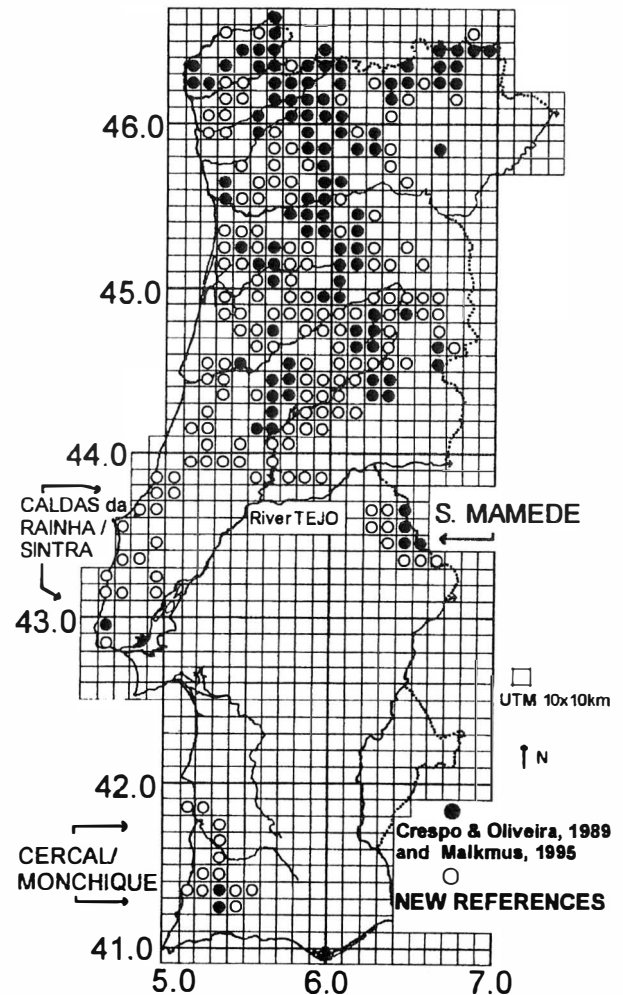


FIG. 1. Sampled area between 1994 and 1996.

new information while only 39% correspond to citations from Crespo & Oliveira (1989) and Malkmus (1995). Within the isolated populations there is an increase of 300% in the known occupied area. In Fig. 3 we present the actual total Iberian distribution of *L. schreiberi*.

NORTHERN AREAS IN PORTUGAL

In previous studies, *L. schreiberi* had only been detected in a continuum from the north of Portugal to the region of Ferreira do Zêzere / Coimbra. In the present survey, it has been possible to extend the range of the species almost as far as Sintra mountain. Although it is relatively abundant in the region of Caldas da Rainha / Montejuento, populations are very fragmented, occurring discontinuously between watercourses, due to increasing human intervention with reduction in habitat availability and quality. Fig. 2 may appear to indicate a continuous distribution from Caldas da Rainha up to Sintra Mountain, but this is not the case due to high fragmentation levels as can be seen in Fig. 4. In this area, from a total of 200 1 x 1 km sampled squares, Marques *et al.* (1995) and our own survey only detected

FIG. 2. Distribution area of *L. schreiberi* in Portugal.

the species in 18 of them, which represents 9% of the total sampled area. Sintra mountain is where *L. schreiberi* populations have the greatest risk of extinction, being detected by Marques *et al.* (1995) and our survey, in only five locations: Marmeleiros stream, Urca stream, lagoons of Monserrate, Monserrate Park and Colares stream, distributed within four 1 x 1 km UTM squares (Fig. 4).

S. MAMEDE

Around this mountain there was also an increase in the known distribution area (Fig. 5) especially to the west and south of the mountain. From a total of 79 UTM 1 x 1 km sampled squares, the species was detected in 49, which represents 62% of the sampled area. The species is common above 600 m, descending to 250 m along the streams of Nisa (north-west), Sever (north), Arronches, Xévorá and Soverete (south). An isolated population also appears along the Abrilongo stream at the south-eastern extremity of the mountain. The species in this region is strongly associated with the presence of the royal fern (*Osmunda regalis*), an indicator of high humidity.

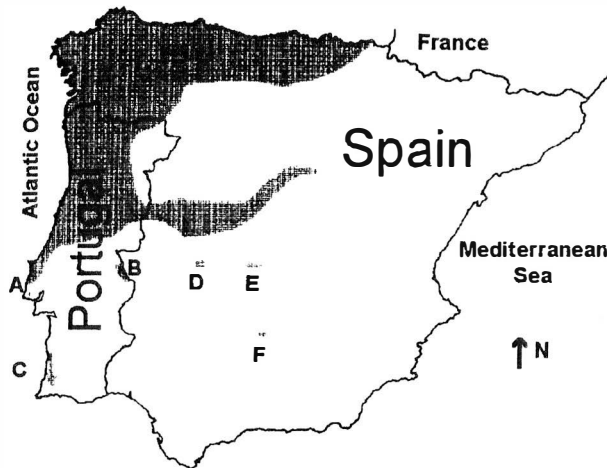


FIG. 3. Iberian distribution area (dark) of *L. schreiberi*. A, Sintra mountain; B, S. Mamede mountain; C, Monchique/Cercal mountains; D, Las Villuercas mountains; E, Montes de Toledo; F, Morena mountain.

MONCHIQUE/CERCAL

Fig. 2 suggests there is a continuum between Cercal and Monchique mountains. Nevertheless there is a high level of fragmentation between the populations (Fig. 6), which are restricted to isolated watercourses and surrounded by unfavorable habitats for dispersion and migration, such as eucalyptus forests or arid agricultural systems. From a total of 121 UTM 1 x 1 km sampled squares the species was detected in 48, which represents 40% of the sampled area. The majority of the observations took place in the higher areas of Monchique mountain (above 400 m), in undisturbed watercourses and sometimes in abandoned agricultural fields. Above 600 m *L. schreiberi* is associated with the presence of *Rhododendron* (*Rhododendrum ponticum baeticum*), which is a bush associated with an Atlantic climate (Lopez, 1991). There was an extension of the known distribution area to lower areas, along the valleys of Aljezur and Seixe streams. We detected also small isolated populations at the Vale das Amoreiras and Seca streams.

The Cercal mountain populations are more fragmented and are completely isolated from each other, with *L. schreiberi* being detected in only three watercourses: Selas and Godins ravines, and Torgal stream. In these watercourses the surrounding biotope is commonly cork oak groves or arid agricultural systems. Populations are small, with fewer than 3700 individuals (Brito *et al.*, unpublished data), and mostly fragmented, making it very likely that they will become extinct if active conservation measures are not taken.

HABITAT

Due to the agricultural utilization of the river banks, the habitat to which the species is restricted is the margins of the watercourses. However, in high altitude areas, where the surrounding habitat of streams is preserved, it is common to observe the species far from the watercourses at distances up to more than 300 m.

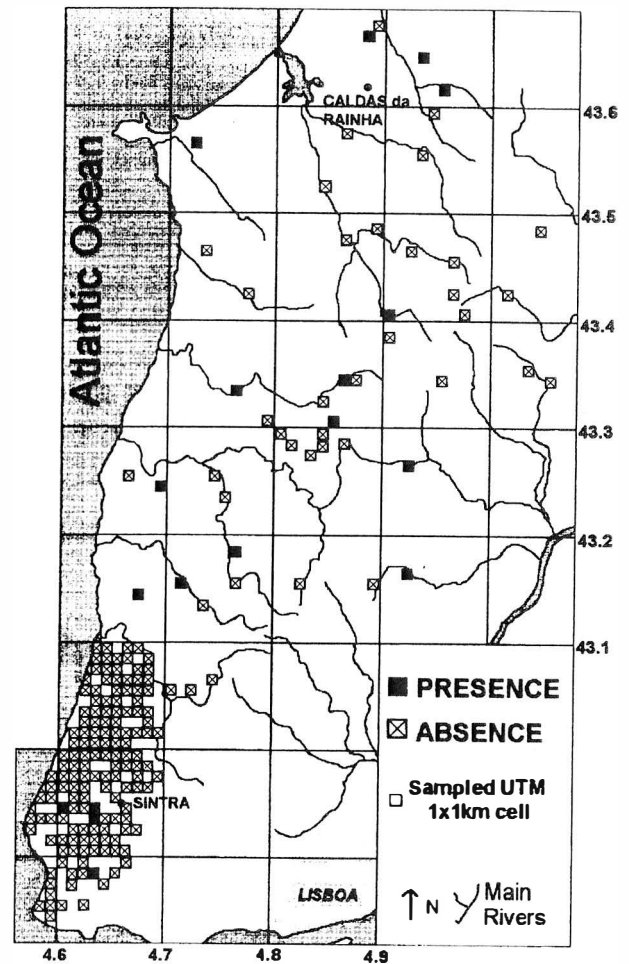


FIG. 4. Distribution area of *L. schreiberi* in the region between Caldas da Rainha and Sintra mountain (Major grid UTM 10 x 10 km).

NORTH OF RIVER TEJO

In Table 2 are presented the five variables in which significant differences ($P < 0.01$) were detected.

Water velocity. The species is only absent from watercourses with no running water; i.e. streams which may dry out in summer.

Water quality. This variable partly indicates, as does water velocity, that the watercourses in which *L. schreiberi* does not appear are those that dry out. Nevertheless the species seems to be absent from polluted watercourses but unexpectedly it is apparently indifferent to very polluted watercourses.

Dominant species in the tree and shrub strata. *L. schreiberi* prefers watercourses in which the tree stratum has Common Alder (*Alnus glutinosa*), White Birch (*Betula pubescens*) and English Oak (*Q. robur*), and the shrub stratum has young Common Alder, Royal fern (*Osmunda regalis*) and Ivy (*Hedera* sp.). All these species only appear in habitats with an Atlantic climate (Lopez, 1991). On the other hand *L. schreiberi* seems to avoid places with Narrow-leaved Ash (*Fraxinus angustifolia*), Poplar (*Populus* sp.), Holm Oak (*Q. ilex*), Holm-shrub (*Q. coccifera*), Cork Oak (*Q. suber*), Olive (*Olea europaea*), Tamarisk (*Tamarix* sp.), Carob (*Ceratonia siliqua*), Mastic (*Pistacia lentiscus*) and

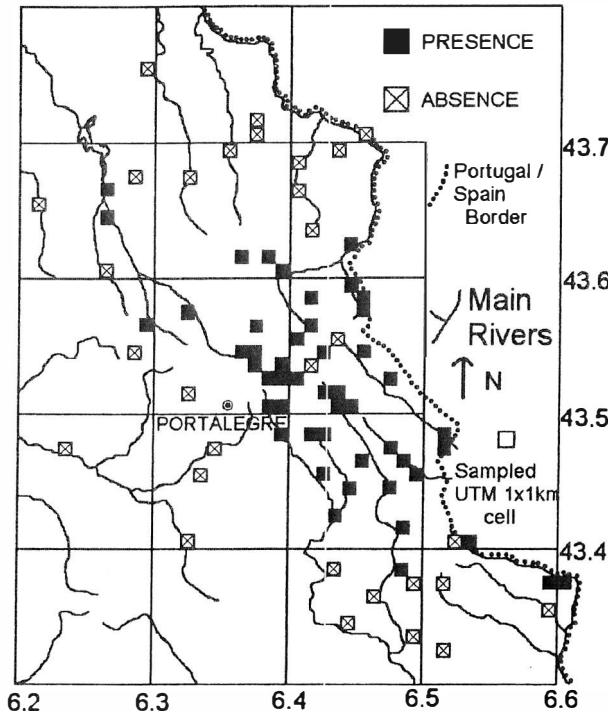


FIG. 5. Distribution area of *L. schreiberi* in the region of S. Mamede mountain (Major grid UTM 10 x 10 km).

Oleander (*Nerium oleander*) in the tree and shrub strata. All these species are typical of Mediterranean ecosystems (Lopez, 1991).

Surrounding biotope. The streams which the species appears to prefer are located in montane agricultural valleys or surrounded by forests of English and Pyrenean oak. The best measure of habitat disturbance, intensity of human activity, was not statistically significant ($\chi^2 = 6.5$, d.f. = 5, $P > 0.01$), which indicates a certain tolerance for human activities with reduced impact on the streams.

ISOLATED POPULATIONS

In Table 3 we present the five variables, for the isolated population of S. Mamede, in which significant differences ($P < 0.01$) were detected. These variables are nearly identical to those for the northern region, which means that even at a smaller scale these variables are those that best explain the habitat selection of the species. The exception is altitude, with the species apparently absent below 250 m. In the Monchique/Cercal population, the only variable that held significant differences was the dominant species in the tree stratum, with results identical to those for the northern and southern Mamede populations.

DISCUSSION

The distribution of this species in Portugal is clearly Atlantic, occurring in all northwestern and practically all northern and central areas. The species does not cross the river Tejo, due to the climatic transition that occurs in this area, with the rainy climate of the north

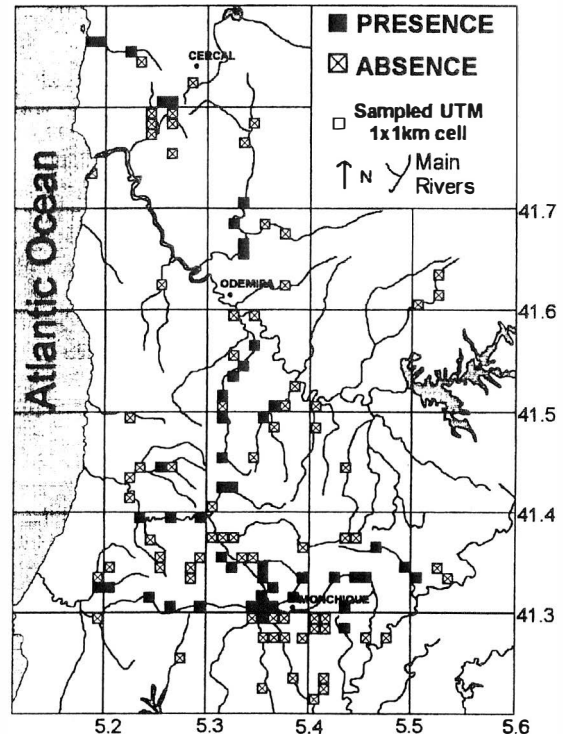


FIG. 6. Distribution area of *L. schreiberi* in the region of Monchique and Cercal mountains (Major grid UTM 10 x 10 km).

being replaced by the dry conditions of the south. The potential area of occurrence of *L. schreiberi* according to Brito *et al.* (1996, submitted b) also confirms these results.

The isolated populations of Monchique and S. Mamede have relatively large distribution areas and are not very fragmented. Nevertheless, the populations of Cercal and Sintra mountains are extremely fragmented and have few individuals (Brito *et al.*, unpubl. data), with Sintra being the most threatened. This population was probably in contact with those in the north until quite recently. However, the post-glacial climatic alterations, combined with increasing human pressure between Sintra and Caldas da Rainha, as all along the Portuguese coast, will have fragmented the populations. Since the population of Sintra does not have the capability to recover by itself, a plan has been established in co-operation with the Sintra-Cascais Natural Park (Marques *et al.*, 1996) to assist its recovery.

Some important habitat characteristics seem to be highly correlated with climate, namely the vegetation type and surrounding biotope. The habitat selection patterns that *L. schreiberi* seems to exhibit appear to be the result of its preference for an Atlantic climate. So the presence of *L. schreiberi* in a given watercourse seems to be more dependent on the climate of that region than on intrinsic characteristics of that watercourse. For example, the species is absent from non-flowing streams and those that are dry. Non-flowing streams will possibly dry out completely in the summer, and this seems to be a restrictive condition for the occurrence of the species.

TABLE 2. Chi-squared results for the variables with significant differences to the north of the river Tejo. *N*, number of samples; Obs, Observed frequency; Exp, Expected frequency; Pre, Preference, which can be positive (+), neutral (0) or negative (-); ** $P < 0.01$; *** $P < 0.001$.

Variable	<i>N</i>	Obs	Exp	Pre
<i>Water velocity</i> **	507			
Zero	128	29	50	-
Medium	136	60	53	+
Fast	217	93	84	+
Very fast	26	14	10	+
<i>Water quality</i> **	507			
Dry	37	2	14	-
Clear	265	111	102	+
Inorganic turbidity	65	26	25	0
Organic turbidity	71	35	27	+
Polluted	52	14	20	-
Very polluted	17	8	7	0
<i>Dominant spp. in shrub strata</i> **	490			
Mediterranean species	10	1	4	-
Brambles	340	118	134	-
Other sps and exotics	14	4	6	0
Marsh sps	40	16	16	0
Unidentified gramineous	27	14	11	+
Atlantic spp.	59	40	23	+
<i>Dominant sps in tree strata</i> ***	433			
Narrow-leaf ash	36	5	13	-
Pine	18	1	7	-
Mediterranean trees	30	7	11	-
Poplars	21	5	8	-
Exotic spp.	25	7	9	0
Orchards	19	7	7	0
Marsh spp.	38	20	14	+
Willows	78	38	29	+
Atlantic trees	168	84	62	+
<i>Surrounding biotope</i> ***	503			
Olive cultures	33	0	13	-
Shrubby areas	44	10	17	-
Diverse pinewoods	36	9	14	-
Cork and holm oak groves	6	0	2	0
Eucalyptus forests	20	6	8	0
Pine woods	30	10	12	0
Urban development	47	18	18	0
Irrigated/dry farmland	35	16	14	0
English/Pyrenean oak forest	10	7	4	+
Montane farmed valleys+pine	83	42	32	+
Montane farmed valleys	159	76	61	+

L. schreiberi appears to prefer those places where the vegetation is composed of species associated with an Atlantic climate. Habitats receiving high rainfall therefore seem to be important, and the species evidently avoids places with Mediterranean influences. The surrounding biotope also reflects the influence of climate on the presence of the species, because montane agricultural valleys, and English and Pyrenean oak forests, are typical of the northern mountain regions.

TABLE 3. Chi-squared results for the variables with significant differences in the isolated population of *S. Mamede*. *N*, number of samples; Obs, Observed frequency; Exp, Expected frequency; Pre, Preference, which can be positive (+), neutral (0) or negative (-); ** $P < 0.01$; *** $P < 0.001$.

Variable	<i>N</i>	Obs	Exp	Pre
<i>Water velocity</i> ***	105			
Zero	36	3	16	-
Medium	23	3	10	-
Fast	29	25	13	+
Very fast	17	15	7	+
<i>Water quality</i> **	105			
Dry	9	0	4	-
Clear	53	36	23	+
Inorganic+organic turbidity	28	8	12	-
Polluted	15	2	7	-
<i>Dominant spp. in tree strata</i> ***	133			
Narrow-leaf ash	30	1	14	-
Mediterranean trees	7	0	3	-
Poplar	5	0	2	-
Marsh spp.	38	20	19	0
Willows	8	2	4	0
Atlantic trees	45	40	21	+
<i>Surrounding biotope</i> ***	101			
Irrigated/dry farmland	35	8	15	-
Cork oak groves	24	6	10	-
Shrubby areas	11	1	5	-
Olive cultures + pine woods	11	8	5	+
Montane farmed valleys	20	20	9	+
<i>Altitude (m a.s.l.)</i> ***	105			
0 - 250 m	23	0	10	-
250 - 500 m	53	21	23	0
> 500 m	29	25	13	+

Nevertheless, Salvador (1988) and Marco (1994) found variations in substratum and vegetation preferences, but these two studies were carried out in small study areas (maximum: 0.5 ha) when compared to our study. It is therefore to be expected that they found selection at the microhabitat level.

Although the presence/absence of *L. schreiberi* in a watercourse is highly influenced by the climate of that region and far less by any habitat component, it is quite clear that the conservation status of the habitat will play an important role. That is, the streams that are located in climatically favourable regions but in which the habitat is highly degraded or completely altered, will not present conditions for the presence of the species.

Polluted watercourses seem also to be negatively correlated with the presence of the species. The unexpected indifference to very polluted watercourses may be explained by the reduced sampling of this level (less than 3.5%) in relation to the other levels, and not by the unimportance of these highly degraded areas. Pollution, either domestic, industrial or agricultural (arable or pastoral), damages the water quality, and although there has not been any specific study of the effect of

polluted water on *L. schreiberi*, it is known that pesticides are easily accumulated in snakes (e.g. Bauerle, Spencer & Wheeler, 1975). Moreover the degradation of water quality reduces insect diversity, which may affect the feeding of lizards (Marco & Pérez-Mellado, 1988). Although *L. schreiberi* normally lives at the margins of watercourses, we observed individuals on top of rocks in the middle of the stream, and even a female under the water (Brito, personal observation). In fact, escape behaviour of this species very commonly involves running to and jumping into the water and seeking shelter among the marginal rocks (Crespo & Oliveira, 1989). So it is to be expected that this lizard may be affected directly by pollution.

The constant presence of water seems to be a crucial factor for this species, for it is usually absent from habitats where there is no running water throughout the year. It is not known at what level the water is important, if it affects the adults physiologically and/or if the development of eggs requires high soil humidity. Physiological studies on the adults and eggs could be made to obtain a better knowledge of this species.

The habitat to which *L. schreiberi* is now mainly confined – the margins of watercourses – is fragile and easily disturbed. Conservation measures for *L. schreiberi* should encompass (1) particular care in every intervention in watercourses especially when destruction of vegetation is involved (regulation of streams, clearing of margins, large dams, etc.); (2) educational campaigns among local communities should be implemented to encourage the preservation of riparian vegetation and the traditional stone walls that surround the watercourses, which are commonly replaced by walls of concrete; and (3) measures to reduce pollution in watercourses should also be implemented.

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REPRODUCTIVE BIOLOGY OF THE SECRETIVE MEDITERRANEAN COLUBRID *MACROPROTODON CUCULLATUS* IN THE SOUTHERN IBERIAN PENINSULA

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We examined 150 museum specimens of *Macroprotodon cucullatus* from the southern portion of the Iberian Peninsula to determine size at sexual maturity, reproductive and fat body cycles, clutch size and hatching period. Males mature at a smaller absolute and relative size than females and, as in Moroccan populations, show a postnuptial spermatogenic cycle. The mating period occurs earlier than that of any other Mediterranean snake species. Females reproduce every two years and there is no correlation between body size and clutch size. The tendency for *M. cucullatus* to have a biennial reproductive frequency and a fixed clutch size is discussed in relation to some features of the ecology of this species, including its burrowing habit, its likely low range of preferred temperatures in relation to epigeous species, and its low feeding frequency.

INTRODUCTION

In European temperate and subtropical areas oviparous female snakes are monoestrous with a rather uniform reproductive cycle. Ovulation occurs in late spring and early summer, and hatching in late August or September (e.g. Bourlière & Petter-Rousseaux, 1955; Saint-Girons, 1982; Naulleau, 1992; Luiselli, Capula & Shine, 1997). Male reproductive cycles of snakes in the same areas, however, are not so uniform. Most of the species demonstrate spermatogenesis during aestivation, with multiplication of spermatogonia beginning in the spring, and spermiogenesis ending in early autumn (e.g. *Coluber viridiflavus*: Bourlière & Petter-Rousseaux, 1955; *Elaphe longissima*: Saint-Girons, 1963; and *Coronella girondica*: Bons & Saint-Girons, 1982). Some species from Mediterranean and arid zones of the southern Palearctic, however, have prenuptial spermatogenesis which begins in late winter or early spring and ends in early summer (*Malpolon monspessulanus*: Cheylan, Bons & Saint-Girons, 1981; *Coluber hippocrepis*: Bons & Saint-Girons, 1982; Pleguezuelos & Feriche unpub. data).

Macroprotodon cucullatus, the sole species of the genus and the smallest Palearctic colubrid, is distributed from southern Palestine to western Morocco, relictually in central (Hoggar) and western (Río de Oro) Sahara, some western Mediterranean islands, and across the southern half of the Iberian Peninsula (Busack & McCoy, 1990). Known details of its natural history are very peculiar: this species displays the lowest feeding frequency among Mediterranean colubrids, and feeds mostly on long-bodied and burrowing prey; it is secretive and fossorial, with skeletal modifications correlated with its fossorial habit (Rasmussen, 1979; Wade, 1988; Pleguezuelos, Honrubia & Castillo, 1994; Pleguezuelos, 1997).

An aestival reproductive pattern, with spermatogenesis from March to October, and spermatozoa overwintering in the vas deferens until the

following spring, has been found in male specimens from Morocco (Saint-Girons, 1963; Bons, 1967). Why spermatogenesis occurs one to two months before other Palearctic colubrids with aestival spermatogenesis (see Saint-Girons, 1982) is unclear and, apart from occasional reports of specimens in captivity (González de la Vega, 1988) or in the field (Escarré & Vericad, 1981), little is known about the female reproductive cycle and its relationship to the male reproductive cycle.

Here we record body size, ontogenetic and seasonal change in gross gonadal morphology, fat body level, and clutch size of *M. cucullatus* in the Iberian population, and present data concerning size at maturity, reproductive cycle in both sexes, fat body cycling, and certain morphological traits of hatchlings.

MATERIAL AND METHODS

We studied 150 specimens of *M. c. ibericus* (84 males, 64 females and two juveniles of undetermined sex) from the southern Iberian Peninsula (36° 00'-39° 40' N, 1° 25'-7° 23' W). The climate in this area is typically Mediterranean, with mild and rainy winters in the west, cool and less rainy winters in the east, and hot, dry summers throughout (Montero & González-Rebollar, 1982). Specimens were obtained from: Museo Nacional de Ciencias Naturales (Madrid), Estación Biológica de Doñana (Seville) and Departamento de Biología Animal y Ecología (Univ. Granada). Data from preserved material were combined with field observations of mating behaviour and hatching data obtained during standardized searches (1988-1993) throughout the eastern portion of the study area.

Snout-vent length (SVL, ± 1 mm) and total body mass (± 0.1 g) were measured. Lengths and body masses were mainly taken from fresh specimens or obtained from labels of museum specimens that were measured fresh; in ten cases (6.7% of the sample size) the measurements were taken from well-preserved mu-

seum specimens. All specimens were dissected. Testis size (longest, median, and shortest diameter, ± 0.1 mm) was recorded in males. Testicular volume (TV) was calculated using the formula for volume of a flattened ellipsoid, $TV = 4/3 \pi abc$, where a = longest radius, b = median radius and c = shortest radius. Because TV increases significantly with SVL, we used the ratio of organ:body size (SVL^3 as measure of body size) as an indicator of male reproductive activity (Seigel & Ford, 1987; Whittier & Limpus, 1996). In this way, the resultant ratio is independent of body size, although at the limit of significance ($r = 0.253$, $n = 55$, $P = 0.057$). To calculate size at maturity in males, we related the volume of the right testis (always anterior and normally the largest) to body size.

The presence and number of oviductal eggs, diameter of the largest ovarian follicle or oviductal egg (± 0.1 mm), and fat body development in females were recorded. We were not able to remove the fat bodies from some museum specimens and scored fat bodies from zero (no traces of fat) to four (a continuous fat layer in the ventral part of the abdominal cavity). Reproductive condition of each female was established by presence of vitellogenic follicles, oviductal eggs, and internal signs of recent oviposition (widened oviducts). For clutch size, we made conservative estimates by counting only shelled oviductal eggs or well-developed vitellogenic follicles. The proportion of gravid females in the sample during the reproductive period served to estimate frequency of reproduction. Appearance of neonates in the field and collection dates for the smallest specimens in collections served to approximate time of hatching. To calculate size at hatching, we consider only young seen or collected during this period with fresh vitellogenic scars. Specimens were sexed in the field following Feriche, Pleguezuelos & Cerro (1993).

RESULTS

Apparently the relative testicular volume (RTV) does not increase with body size in specimens with $SVL < 240$ mm. Beginning at 240 mm SVL, there is a testicular growth that implies an 8- to 12-fold increase in RTV (Fig. 1). Therefore, we considered males exceeding this size to be sexually mature. The largest male *M. cucullatus* recorded in the Iberian Peninsula measured 475 mm SVL (present data), and males mature at about 50% of maximum length. Largest specimens ($SVL > 370$ mm) have a lower RTV (mean $\pm$ SD = 0.55 ± 0.26 , $n = 6$) than smaller mature specimens ($240 < SVL < 370$ mm; mean $\pm$ SD = 1.24 ± 0.64 , $n = 39$; Mann Whitney *U*-test, $P = 0.002$; Fig. 1). This testicular involution is only apparent, because four out of the six largest specimens were collected outside the testicular recrudescence season.

Testicular recrudescence began in early May with peak volume being achieved by late September. Testis volume decreased sharply during October and regressed testes were observed during autumn and late winter, and presumed, but not observed, during mid-

winter (RTV in September versus October + November + December; *t*-test, $t = 3.95$, $P = 0.01$; Fig. 2). Copulation was observed in the field as early as 15 March. In captivity, mating also occurs in March in specimens from the south-western Iberian Peninsula (J. P. González de la Vega, pers. comm.). The male reproductive cycle is seasonal and annual progression of the RTV indicates that *M. cucullatus* has an aestival spermatogenic cycle.

The smallest *M. cucullatus* female that showed signs of reproductive activity (vitellogenic and well-developed follicles with high fat body level) was 280 mm SVL (Fig. 3). A female of 302 mm SVL from the south-west of the Iberian Peninsula deposited two eggs (González de la Vega, pers. comm.). Therefore, we consider that females mature at about 280-300 mm SVL. No female with a $SVL < 280$ mm collected during the period of reproductive activity (late spring or early summer, $n = 5$) contained either enlarged vitellogenic follicles or oviductal eggs. Each of the small group of females with a SVL between 255-275 mm collected during early summer and autumn with follicles 1.1-6.6 mm in length (Fig. 3) was non-reproductive. The largest female *M. cucullatus* recorded in the Iberian Peninsula measured 409 mm SVL (present data), and females mature at about 70% of maximum length.

As expected, the female reproductive cycle is also seasonal. Vitellogenesis begins in late March, in follicles 4 mm in diameter. Ovulation occurs from April to May, and females are gravid between early May and early July (Fig. 4). Post-reproductive females (distended oviducts) were observed from early July. We estimate oviposition in the field to occur between June and early July. We found a few specimens with follicles > 4 mm in length in summer (Fig. 4), but do not interpret this as post-reproductive vitellogenesis, because they were atretic.

The mean number ($\pm$ S.D.) of oviductal eggs was 4.16 ± 0.75 (range = 3-5, $n = 6$), and we found no correlation between female SVL and the number of oviductal eggs ($r = 0.26$, $n = 6$, $P = 0.62$). Six out of the twelve (50%) sexually mature females in the reproductive period (May to early July) contained oviductal eggs or enlarged follicles. In order to test any bias by the effect of body size, we compared SVL in mature but

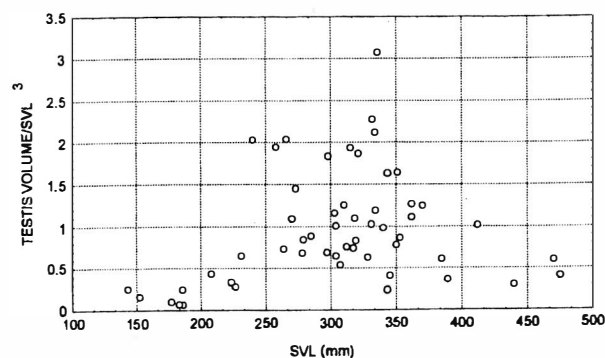


FIG. 1. Gonadal activity of male *M. cucullatus* as indicated by relative testicular volume versus body length ($n = 55$).

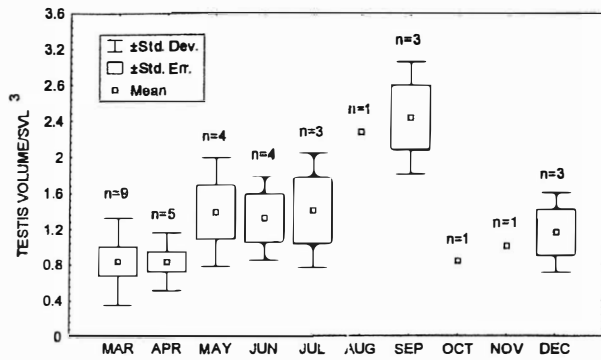


FIG. 2. Relative testis volume of sexually mature male *M. cucullatus* (SVL > 240 mm, $n = 34$) in relation to calendar year.

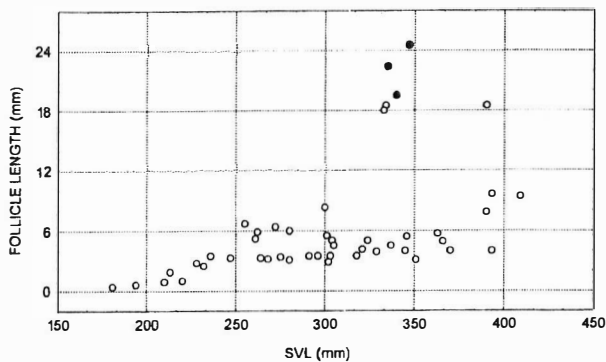


FIG. 3. Gonadal activity of female *M. cucullatus* as indicated by length of the largest previtellogenic follicles (< 3-4 mm; open circle), vitellogenic follicles (open circle) and oviductal eggs (solid circle) versus body length ($n = 48$).

non-reproductive females (mean $\pm$ SD = 350.0 ± 28.1 mm, $n = 6$) and reproductive females (mean $\pm$ SD = 358.0 ± 34.1 mm, $n = 6$), finding no difference (Mann Whitney U -test, $P = 0.4$). The low frequency of reproductive females suggests a biennial reproductive cycle.

We noted no significant monthly differences in fat body level in reproductive females (Kruskal-Wallis test, $H_{6,28} = 9.50$, $P = 0.15$; October to February omitted because of small sample size; Fig. 5).

First hatchlings were recorded in the field on August 15, and more appeared in both field and museum collections toward late September. Hatchling SVL varied from 123 to 166 mm (mean $\pm$ S.D. = 146.5 ± 11.8 ; $n = 15$) and body mass was between 1.4 and 2.5 g (2.0 ± 0.3 g; $n = 15$). Taking length at maturity to be 240 mm SVL in males and 280-300 mm SVL in females, there is an increase between 1.6 and 1.9 times the hatching length on reaching maturity for males and females respectively.

DISCUSSION

Male *M. cucullatus* mature at a smaller absolute and relative size (SVL at maturity versus maximum SVL) than females, despite the lack of sexual dimorphism in body size found by Feriche *et al.* (1993) working with the same data set used at the current study. In a recent

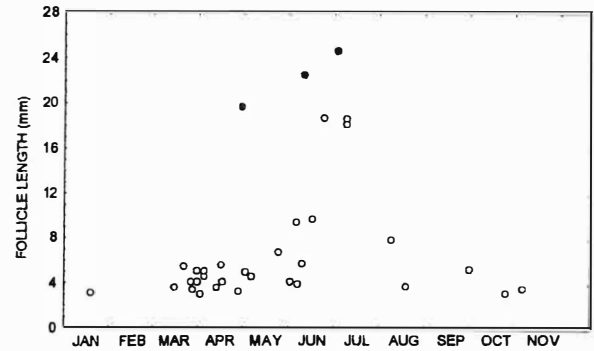


FIG. 4. Length of the largest follicle (open circle) and oviductal egg (solid circle) of sexually mature female *M. cucullatus* (SVL > 280 mm, $n = 34$) in relation to calendar year. The two females from early June with follicles about 9 mm in length were non-reproductive (very low level of abdominal fat).

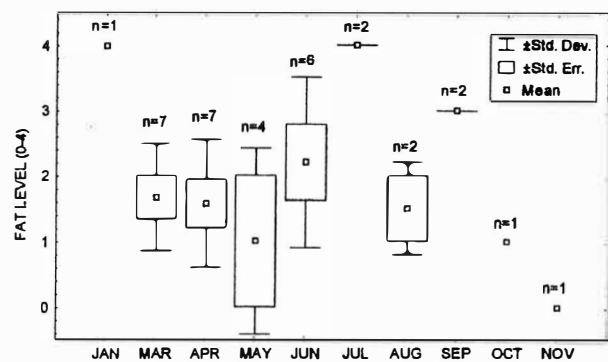


FIG. 5. Abdominal fat level for reproductive female *M. cucullatus* ($n = 33$).

review, Parker & Plummer (1987) also found that males of about two-thirds of the temperate snake species analysed (sample size of 58 species) matured at a smaller body size than did females. Cost to males of early maturity is lower than that for females. Moreover, females appear to delay maturity because fecundity is proportional to body size (Shine, 1978; Schwaner & Sarre, 1988).

Our data indicate that the reproductive cycle of male and female *M. cucullatus* in the southern Iberian Peninsula begins in spring, as it does in Morocco (Bons, 1967). This pattern appears in females of all snake species from the Western Palearctic (e.g. Goddard & Spellerberg, 1980; Naulleau, 1992; Baron, Ferriere, Clobert & Saint-Girons, 1996; Luiselli *et al.*, 1997). From the geographic distribution of *M. cucullatus*, a prenuptial spermatogenic cycle would be expected (as in *Malpolon monspessulanus* and *Coluber hippocrepis*, Mediterranean colubrids with a similar distribution; Cheylan *et al.*, 1981; Pleguezuelos & Feriche, unpubl. data). However, as in other colubrid species having a more northerly Palearctic distribution (see data in Introduction), this species has a postnuptial spermatogenic cycle (Bons, 1967). A previous study revealed *M. cucullatus* in the western Mediterranean to have the lowest frequency of specimens with gut con-

tents among Mediterranean colubrids (24.6% of individuals contained food remains in the whole gut; Pleguezuelos *et al.*, 1994). In the current data set, only 14.7% of the mature females ($n = 34$) and 14.0% of all females pooled ($n = 50$) had prey in their stomachs. In our previous study we suggested that this species had low energy requirements, as is expected in reptiles that live under rocks or lead a fossorial life style (Kamel & Gatten, 1983). Moreover, if *M. cucullatus* dwells under rocks and burrows (Pleguezuelos, 1997), it follows that it must thermoregulate more slowly than do epigeous species. Thus, this species would have a postnuptial spermatogenic cycle and a lengthy spermatogenesis period because it is likely that low energy specialization precludes it from developing the entire spermatogenic cycle in a short period. This contrasts with other Mediterranean species, normally epigeous and non-secretive, with a prenuptial cycle in which spermatogenesis occurs rapidly (Cheylan *et al.*, 1981; unpubl. data from the authors). Temperature is the main environmental factor related to testicular activity in temperate reptiles (Marrion, 1982) and the onset of the spermatogenic cycle in *M. cucullatus* begins with the warmer temperatures of mid-spring, and extends through the hottest months of the year.

The mating period of *M. cucullatus* occurs earlier (see also González de la Vega, 1988) than in any other Mediterranean snake species (i.e. mid-May in *Coluber viridiflavus*: Saint-Girons, 1982; April-June in *Coronella girondica*: González de la Vega, 1988; June in *Elaphe scalaris*: Blázquez, 1993; May in *Natrix natrix*: Luiselli *et al.*, 1997). In the study region, activity differed between sexes, with a higher peak in male activity detected in early spring, but at the limit of significance (Yate's corrected $\chi^2 = 3.76$, $df=1$, $P = 0.052$; Pleguezuelos & Feriche, unpubl. data). This pattern is probably associated with the mating period. Male specimens from Morocco, on emerging from overwintering (toward mid-March), have reproductive ducts full of spermatozoa (Saint-Girons, 1963; Bons & Saint-Girons, 1982). If *M. cucullatus* proved to be a low energy specialist, copulation would be accomplished early in the year because females would then have a longer period for completing vitellogenesis, ovulation and shelling than other Mediterranean snakes. Although the long period during which we found females with oviductal eggs (75 days; Fig. 4), supports this suggestion, it is only tentative, because the scarcity of mature females during early spring (Fig. 4) precludes us from establishing the real length of the vitellogenesis period.

Breeding frequency is among the most important yet least understood aspects of snake reproductive biology (Seigel & Ford, 1987). Most studies on snake reproductive ecology have used the proportion of gravid females in the population to estimate the frequency of reproduction (see review in Seigel & Ford, 1987). Although interpretations about reproductive cycles based on museum specimens must be treated cautiously, half the *M.*

cucullatus females in collections from the southern Iberian Peninsula had enlarged follicles and oviductal eggs in the appropriate reproductive period. Reproductive frequency, based on percentage of reproductive snakes, could be biennial in female *M. cucullatus*.

This method of estimating the reproductive frequency has recently been criticized (Bonnet & Naulleau, 1996; see also Martin, 1993). Females undergoing vitellogenesis and pregnancy may require body temperatures higher than non-reproductive females, and Bonnet & Naulleau (1996) found that reproductive *Vipera aspis* females basked more frequently than non-reproductive *V. aspis*, resulting in different catchability among females; sampling bias can strongly affect breeding frequency estimates in snakes. Nevertheless, we believe that the difference in catchability found in *V. aspis* does not apply to the species here studied. *M. cucullatus* spend much of the time sheltering under stones, at least in the south-eastern Iberian Peninsula (Pleguezuelos *et al.*, 1994), and only rarely bask, even during the reproductive period (Busack & McCoy, 1990; Pleguezuelos, 1997). Most specimens in collections have been found under stones (from personal observations and notes on the labels of the specimens), and thus the probabilities of finding reproductive and non-reproductive females may be the same. Anyway, if gravid females were selectively collected because they were more sedentary and obvious, then a reduction in reproductive frequency in the sample is likely to reflect a more severe reduction in frequency in the total population (G. Shea, pers. comm.).

In a survey of 38 species and populations of colubrids, Dunham, Miles & Reznick (1988) found that most species reproduced annually, and only 8% of species were distinctly biennial in breeding frequency. Moreover, *M. cucullatus* SVL at maturity and mean SVL of adult females are lower than the mean for the same life history traits for biennially reproducing snakes (613.5 mm and 747.7 mm, respectively, in a review of 30 species; Dunham *et al.*, 1988), and any known colubrid with biennial or triennial reproduction (see Dunham *et al.*, 1988). These traits of its reproductive ecology could be related to the low-energy specialization here suggested for this species. As it seems that female reproductive frequency essentially depends on resource availability (Andren & Nilson, 1983; Seigel & Ford, 1987; Whittier & Crews, 1990), and adult female *M. cucullatus* are food limited (Pleguezuelos *et al.*, 1994; present data set), they may be unable to recoup energy in less than one year following parturition. Constraints on activity and foraging may limit the reproductive capacities of females (Dunham *et al.*, 1988). The presence of non-reproductive adult females in the sample studied could explain our not having found either monthly differences in fat body levels or an association of vitellogenesis with the decline in fat body levels that appears in other temperate snakes (Diller & Wallace, 1984; Hailey & Davies, 1987).

Female fecundity was low, but within the range of other small colubrids from temperate areas (Seigel & Ford, 1987; Dunham *et al.*, 1988). Scattered data on the reproduction of this species indicate a clutch size of three for one female just to the north of the study area (Escarré & Vericad, 1981) and two to six for specimens in captivity from the south-west of the Iberian Peninsula (González de la Vega, 1988). A positive correlation between female SVL and clutch size is common among snakes (Ford & Seigel, 1989; Dunham & Miles, 1985). The lack of correlation in *M. cucullatus* between female size and female fecundity may be an artifact of sample size, and may also be related to the narrow band of body sizes at which females appear to be reproductive. If we pool our data with data gathered by González de la Vega (pers. comm.) from the south-west of the Iberian Peninsula, and data gathered by us in populations from similar climatic areas in north-western Africa, we still fail to find any correlation between female body size and clutch size ($r = 0.38$, $n = 12$, $P = 0.21$). Low correlation between female SVL and fecundity reflect the trend towards a fixed clutch size. Invariant clutch size is normally found in fossorial (H. Saint-Girons, in Agrimi & Luiselli, 1994) and prey-limited snakes (Shine, 1988). *M. cucullatus* fits these characteristics because its morphology (Rasmussen, 1979) and ecology (Pleguezuelos, 1997) places it among the most fossorial snakes in the Western Palaearctic, and its likely sit-and-wait foraging habit places it among those terrestrial snakes with the lowest feeding frequency (Pleguezuelos *et al.*, 1994). Moreover, when grouped by reproductive frequency, foraging mode, distribution, etc., the lowest values in a regression analysis of mean clutch size on female SVL at maturity are found in biennially reproducing snakes (Dunham *et al.*, 1988).

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ORIGIN OF THE YELLOW-BELLIED TOAD POPULATION, *BOMBINA VARIEGATA*, FROM GÖRITZHAIN IN SAXONY

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Analysis of variation at four allozyme loci demonstrated that a population of the yellow-bellied toad in Göritzchain (Germany) does not represent an easternmost relict population, but is descended from Romanian toads introduced there about 15 years ago.

INTRODUCTION

Application of electrophoretic methods to studies of protein variation in natural populations has uncovered unexpectedly high levels of protein variation (Lewontin, 1991). Electromorphs (or their underlying alleles) are rarely uniformly distributed across a species range and often form complex geographic patterns. The study of genetic relatedness of populations together with analysis of geographic patterns can provide cues about the history of the populations (Avice, 1994). Regional differentiation also means that local populations bear "genetic tags" which provide a means of tracing the origin of populations suspected of being introduced.

The European fire-bellied toads, *Bombina bombina* and *B. variegata*, are closely related species with parapatric distributions, which hybridize along their contact zone (Arntzen, 1978; Gollmann, 1984, 1987; Gollmann, Roth & Hödl, 1988; Piálek, 1992; Szymura, 1976, 1988, 1993). Despite hybridization, the species retain their identities and can be easily diagnosed away from a narrow hybrid zone by a variety of morphological, anatomical, behavioural and molecular characters (Szymura & Barton, 1991; Szymura, 1993; Nürnberger *et al.*, 1995; MacCallum *et al.*, 1998). Electrophoretic studies of enzyme variation and mitochondrial DNA showed that both species are differentiated into regional groups (Szymura, 1988). Of the two species, *B. variegata*, which inhabits higher mountains of southern and western Europe as well as the Carpathian Mountains, is more subdivided into geographic groups. Four such groups, corresponding in part to subspecific categories, have been distinguished in *B. variegata*: the Italian group (*B. v. pachypus*), the Balkan group (*B. v. scabra*), the western group and the Carpathian group. The Carpathian group seem to be heterogenous, with south-eastern populations possessing alleles absent over the rest of the range. The western and the Carpathian populations correspond to *B. v. variegata*. The pattern of variation observed in *B. variegata* at allozyme loci is complicated. It involves areas of either high variation or monomorphism, relatively narrow transition zones from one type to the other or broad clinal variation. In any case, the knowledge of geographic variation pattern in *B. variegata* is sufficient to identify the geographic origin of any unknown sample taken from anywhere in Europe with considerable precision.

In this paper I provide information on the origin of *B. variegata* from Göritzchain near Burgstätt in Saxony (Fig. 1). The yellow-bellied toads inhabiting this locality are suspected of being introduced. They could be descendants of some 20 toads collected in the Fagaras Mountains in Romania and released in 1978 or 1982 at a site some 500 m from the sandpit in which two permanent ponds and several temporary pools, all inhabited by *B. variegata*, are located (Tolke, 1996). An alternative explanation for the presence of *B. variegata* in Göritzchain implies that this population is autochthonous and represents an easternmost relict of a wider distribution of *B. variegata* in the past, in Saxony and Thuringia. Schiemenz (1980, 1981) reported *B. variegata* from the vicinity of Gera, about 50 km west of Göritzchain. Unfortunately, near Gera *B. variegata* have gone extinct (Pontius, 1985).

These two alternative scenarios provide easily testable hypotheses. Depending on the scenario, the yellow-bellied toads from Göritzchain could be genetically similar either to western, or to Romanian toads. A third possibility is that the Göritzchain population might be of mixed descent, and therefore genetically intermediate, with proportions of alleles reflecting relative contribution of the two separate populations.

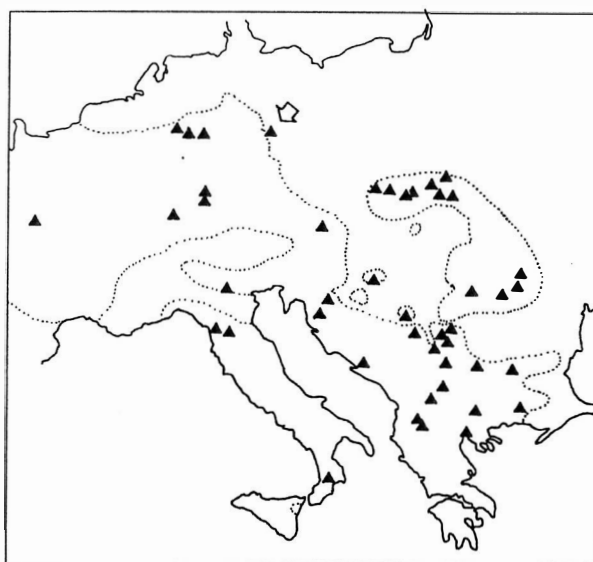


FIG. 1. Location of *B. variegata* samples in Europe. The Göritzchain sample is marked with an arrow. The dotted line indicates the species range.

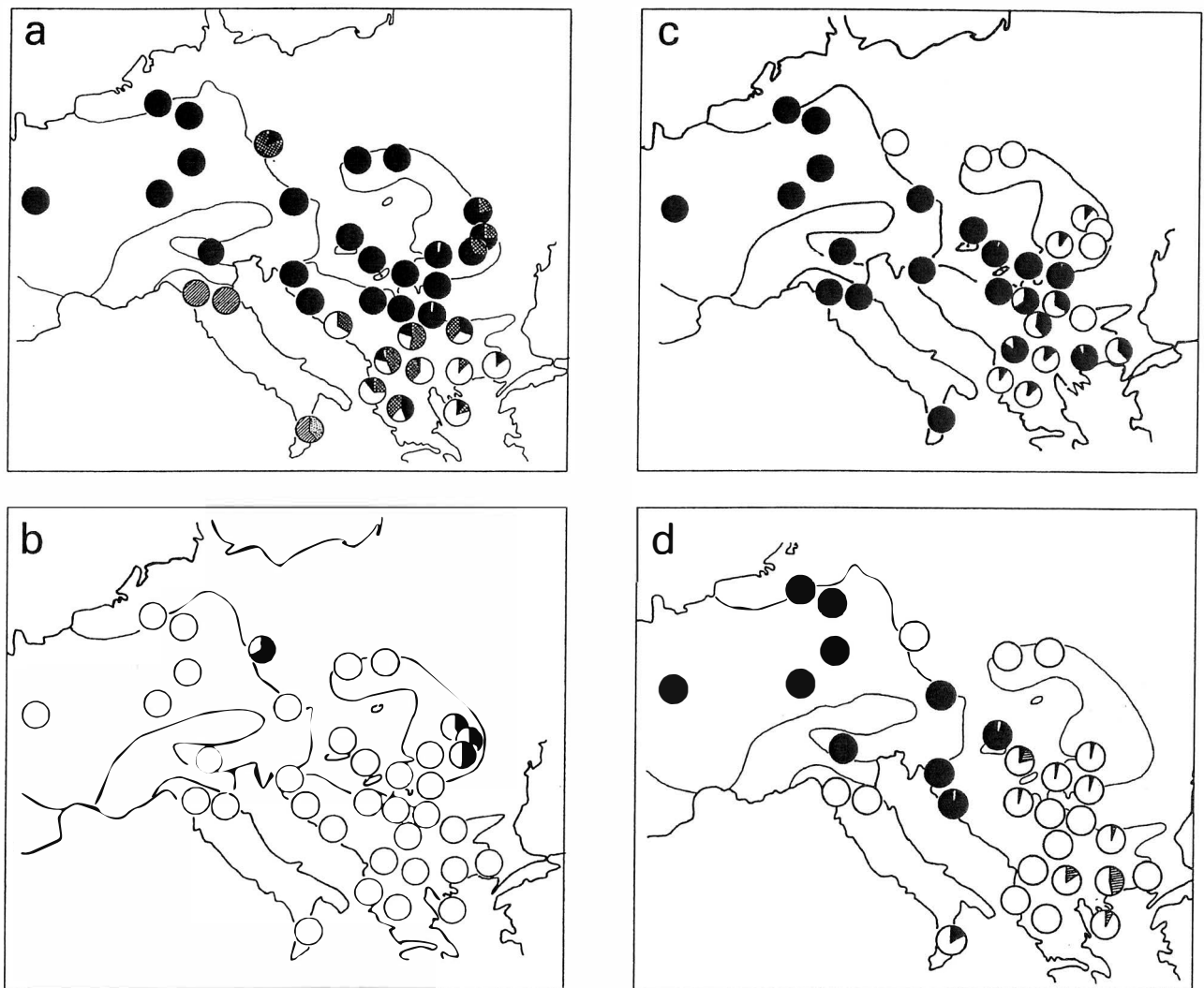


FIG. 2. Geographic patterns of allozyme variation at four enzyme loci in *B. variegata* samples from Europe. (a) Ldh-1: black - Ldh-1 F, crosshatched - Ldh-1 M, white - Ldh-1 R, hatched - Ldh-1 I, stippled - Ldh-1 IR; (b) Mdh-1: black - Mdh-1 M, white - Mdh-1 F; (c) Idh-1: black - Idh-1 F, white - Idh-1 S; (d) Aat-1: black - Aat-1 S, white - Aat-1 M+F, hatched - Aat-1 R. Thin lines indicate the species boundary.

Allozymes can be studied in extracts prepared from amputated toes (Szymura & Farana, 1978). This method has the advantage of not having destructive effects on a local population, since the animals are released in the place of capture. In addition they are permanently marked and a photograph of their belly pattern provides an easy means of identification in future studies.

METHODS

Nineteen adult *B. variegata* (9 males, 10 females) were captured in June 1994 at the sandpit south of Göritz and anaesthetized in 1% solution of MS 222 (Sandoz). The toads were photographed and assigned numbers, and a single middle hind-leg toe was amputated. The toes were frozen on dry ice and later transported to Cracow. All animals recovered from anaesthesia and were released into the ponds.

Toes were homogenized in distilled water, and extracts were subjected to horizontal starch gel electrophoresis (Szymura, 1976, 1983, 1995; Szymura & Farana 1978) along with reference samples from

Switzerland, Poland and Romania. These reference samples represent western, Polish and south Carpathian groups of populations. Variation of the following loci was studied: lactate dehydrogenase (Ldh, 2 loci), isocitrate dehydrogenase (Idh, 2 loci), malate dehydrogenase (Mdh, 2 loci) and aspartate aminotransferase (Aat, 2 loci). These loci were chosen, because they represent loci with diagnostic electromorphs (alleles) distinguishing the western populations from the Romanian groups (Szymura, 1988, 1993). Aspartate aminotransferase was studied in a lithium buffer, pH=7.2. Under these conditions, the F-electromorph (Szymura, 1983, 1988) proved heterogeneous and resolved into two forms: Aat-1 F and Aat-1 M. The latter form had slightly greater mobility than Aat-1 S. The Aat-1 F and Aat-1 M are pooled in Fig. 2d, since their distribution in south-eastern Europe is not known.

RESULTS

Of the eight investigated loci four were monomorphic and did not show any variation: Ldh-2,

Mdh-2, Idh-2 and Aat-2. The other four loci, namely Ldh-1, Mdh-1, Idh-1 and Aat-1, showed variation either in the Göritzchain sample or in the reference samples (Fig. 2, Table 1, 2).

The Ldh-1 locus had two electromorphs in the Göritzchain sample, M and F, with respective frequencies of 0.816 and 0.184. The Mdh-1 locus had two alleles, M and F. The M allele predominated; its frequency was 0.737. All individuals were monomorphic for the Idh-1 S allele. The Aat-1 locus was polymorphic

with two alleles, M and F, with respective frequencies of 0.222 and 0.778. All three genotypes expected under diallelic variation were observed in the sample.

Genotypic distributions at all three polymorphic loci: Ldh-1, Mdh-1 and Aat-1, conformed to the Hardy-Weinberg equilibrium.

DISCUSSION

The western group of *B. variegata* is characterized by the following combination of electromorphs: Ldh-1 F, Mdh-1 F, Idh-1 F and Aat-1 S, and thus the Göritzchain population was expected to have the same combination of alleles under the hypothesis of an autochthonous origin (Fig. 2). This hypothesis has to be rejected, since the sample had a different genetic composition. Ldh-1 had two alleles, M and F; Mdh-1 also had two alleles, M and F; Idh-1 was monomorphic with the S allele; and Aat-1 had 2 alleles, M and F. All four loci provide strong and consistent evidence against the null hypothesis.

Suppose the Göritzchain population is of mixed ancestry and foreign alleles were incorporated following mating between local and released yellow-bellied toads from some unspecified population in 1978 or 1982, i.e. 12-16 years prior to the sampling date. If this is the case, the presence of Ldh-1 M and Mdh-1 M alleles indicate an enriched local gene pool. These alleles have, however, higher frequencies than the supposedly local Ldh-1 F and Mdh-1 F alleles (Table 2), so considerable replacement must have taken place. The same hypothesis would also imply that local alleles at the other two loci, Idh-1 F and Aat-1 S, were either completely replaced with foreign Idh-1 S and Aat-1 M/F or their frequencies reduced below detectable frequency $1/38 = 0.026$.

Let us consider the time scale required for this proposition. Generation time in *B. variegata* is not

TABLE 1. Genotypes of the Yellow-bellied toads from Göritzchain.

Number	Ldh-1	Mdh-1	Idh-1	Aat-1
1	MF	MF	S	M
2	M	M	S	MF
3	M	MF	S	F
4	MF	MF	S	MF
5	MF	MF	S	MF
6	M	MF	S	F
7	M	M	S	MF
8	MF	MF	S	F
9	M	MF	S	F
10	M	M	S	F
11	M	M	S	F
12	M	M	S	F
13	M	MF	S	F
14	MF	M	S	MF
15	M	MF	S	F
16	M	M	S	F
17	MF	M	S	F
18	MF	M	S	MF
19	M	MF	S	-

TABLE 2. Allele frequencies at Ldh-1, Mdh-1, Idh-1 and Aat-1 in the sample from Göritzchain, and in the reference samples from Romania (Tiniovul Mohos, Predeal, Bilea Valea), Western Europe and Poland. Abbreviations: *n* - number of individuals, NS - not studied. 1, samples pooled from seven localities (cf. Szymura, 1988). 2, samples from the Western Carpathians, seven localities (Szymura, 1988).

		Göritzchain	Romania			W.Europe ¹	Poland ²
Locus		<i>n</i> =19	T.Mohos <i>n</i> =15	Predeal <i>n</i> =12	B.Valea <i>n</i> =23	<i>n</i> =90	<i>n</i> =245
Ldh-1	M	0.816	0.333	0.375	0.084	-	-
	F	0.184	0.667	0.625	0.916	1.000	1.000
Mdh-1	M	0.737	0.467	0.500	-	-	-
	F	0.263	0.533	0.500	1.000	1.000	1.000
Idh-1	S	1.000	0.900	1.000	0.870	-	1.000
	F	-	0.100	-	0.130	1.000	-
Aat-1	S	-			0.02	1.000	-
	M	0.222	NS	NS	-	-	0.562
	F	0.778			0.98	-	0.438

known with precision, but it is likely that 3 years (two hibernations) is the shortest this could be. Assuming that the introduction took place 16 years ago and *B. variegata* successfully mated in the same year, five generations of toads have been produced since then. All the studied *B. variegata* were fully mature individuals, and many of them were very large, at least 3 years old and more likely to be older than 5 years. Allele frequency substitution must therefore have taken place in a shorter time, in four or fewer generations. This is highly unlikely, unless the local autochthonous population was very small, say just a few individuals. It is believed that about 20 individuals were released in Göritzchain (cf. Tolke, 1996). If the local population consisted of only 2, 3, 4, or 5 toads, and they mated randomly with the newcomers, allele frequencies typical of the western group at either *Idh-1* or *Aat-1* locus in the next generation would be 0.091, 0.130, 0.167 and 0.200 respectively. Note that even such a small population consisting of three individuals only, a hardly viable and sustainable population, would transmit a sizeable proportion of the local alleles to the next generation. The local alleles would be difficult to replace within four generations if the mating was random. Complete replacement of local alleles would have to take place independently at *Idh-1* and *Aat-1* loci, along with an increase of alien M alleles at two other loci, *Ldh-1* and *Mdh-1*. This is a highly unlikely event given independent assortment of the loci (Szymura & Farana 1978, Szymura, 1995). Therefore the hypothesis suggesting mixed ancestry of the Göritzchain *B. variegata* has to be rejected.

The source of the population therefore seems to be Romania. Even without prior knowledge of a possible source for the Göritzchain *B. variegata*, the sample's origin could be located from knowledge of the variation pattern at *Ldh-1* and *Mdh-1* loci (Fig. 2). The *Ldh-1* M allele is present in the Balkans and eastern Carpathians only. *Mdh-1* M is a very localized variant observed in the southeastern Carpathian Mountains only. It is this allele which provides a decisive clue to the source of introduced *B. variegata*. The results of the genetic analysis are consistent with the expectation that the Fagaras Mountains was the area in which the ancestors of the yellow-bellied toads inhabiting Göritzchain today were collected.

CONCLUSIONS

Electrophoretic analysis of variation at four enzyme loci demonstrates beyond any doubt that the yellow-bellied toads found in Göritzchain are descended from introduced Romanian *B. variegata*. Although this population may not deserve special protection status under the present law, it offers a unique opportunity to study dispersal and colonization of unoccupied territory. The area could thus offer an unintentional experiment to study population dynamics of species in a modified habitat subject to high human pressure, an environment in which most amphibian species either live or will have to live in the future. The Göritzchain

population possesses several enzyme polymorphisms which could help to assess the impact of inbreeding on the loss of genetic variation.

ACKNOWLEDGEMENTS

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EGG GELATINOUS MATRIX PROTECTS *AMBYSTOMA GRACILE* EMBRYOS FROM PROLONGED EXPOSURE TO AIR

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Desiccation during water recession at oviposition sites can be a major cause of embryo death in clutches of aquatic amphibians. However, aquatic eggs of the north-western salamander, *Ambystoma gracile*, can survive out of water for at least 30 days in the field. Females lay eggs surrounded by a firm jelly matrix which presumably protects embryos from desiccation. In a field study, embryos from clutches located either in or out of the water showed no differences in size or stage at hatching. In laboratory experiments, where genetic effects were controlled, air exposure had no effect on embryo survival rate, size or stage at hatching. The ability of *A. gracile* to survive prolonged droughts or water recession during embryonic development may be shared by other amphibians.

INTRODUCTION

Desiccation of aquatic habitats by drought, drainage, or abstraction for irrigation, could contribute to population declines of some amphibian species (Stebbins & Cohen, 1995). Specifically, decreasing hydroperiod at oviposition sites can be a major cause of death to developing embryos. Given that temperature accelerates embryonic and larval development (Brown, 1976; O'Hara, 1981), many species select warm oviposition sites, such as shallow water (Syse, 1975). However, clutches near the surface may have a higher risk of death during pond or stream desiccation.

The gelatinous egg envelopes of many amphibians can help protect the eggs against mechanical damage, predation, pathogens, and other damaging agents (Semlitsch, 1988; Waldman & Ryan, 1983; Ward & Sexton, 1981). Moreover, eggs in globular masses are better insulated and less likely to freeze than individual eggs or clumps (Zweifel, 1968). Arboreal egg masses of some tree frogs are encased in leaves which protect the eggs from desiccation. These clutches have egg-less capsules that provide water for the embryonated eggs (Pyburn, 1980). Some terrestrial and arboreal foam nests have dry outer surfaces that protect eggs from dehydration (Coe, 1974). In many terrestrial salamanders, females provide protection against dehydration of eggs (review in Jaeger & Forester, 1993). However, aquatic amphibian clutches are usually susceptible to dehydration when they become exposed to air. Forester & Lykens (1988) showed that communal egg deposition in *Rana sylvatica* can partially protect embryos from desiccation in aquatic clutches that became completely exposed to air. Nyman (1937) also found that aggregative egg deposition and the gelatinous matrix in *Ambystoma maculatum* can reduce desiccation in stranded egg masses. However, we have found no previous reports of significant survival of single amphibian clutches subjected to prolonged exposure to air. Our field observations suggest that egg masses of the Northwestern salamander (*Ambystoma gracile*) may be able to survive prolonged air exposure because

its eggs are surrounded by a thick and firm extracellular jelly matrix. Eggs are deposited in globular masses 80 to 150 mm in diameter and ova are 1.5 to 2.5 mm in diameter. Clutch size varies from 40 to 270 eggs (Nussbaum *et al.*, 1983) and volume of jelly per egg is approximately 12.5 cm³.

Ambystoma gracile is an aquatic-breeder, and females attach clutches to branches in relatively shallow water, usually away from contact with other clutches (Nussbaum *et al.*, 1983; Stebbins, 1954). Breeding occurs from January to March in lowland areas, when the water temperature is relatively low. Embryos of *A. gracile* have a relatively long developmental time (30 to 60 days) and can tolerate relatively low water temperatures (Brown, 1976). Slow embryonic development may make *A. gracile* clutches susceptible to air exposure if hydroperiod decreases. To test the effect of air exposure on embryo survival rate, we conducted two laboratory experiments. These experiments were used in combination with field observations of clutches that became naturally exposed to air.

MATERIAL AND METHODS

FIELD OBSERVATIONS

We made field observations and conducted experiments from 20 February to 26 April 1996 at a pond near Fall Creek (Lincoln County, Coast Range, Oregon, elevation 200 m). Water depth receded more than 1 m from 20 February to 30 March. Between 16 and 19 March, 20 *A. gracile* clutches became completely exposed to air when embryos were at Harrison developmental stages 30-35 (Harrison, 1969). On 19 March, 10 of the 20 exposed clutches were selected at random and immersed in the pond water. We left the other 10 clutches out of water, either naturally stranded on land or suspended from branches. Water level in the pond never rose again to the level of the clutches located out of the water.

On 8 April (after at least 20 days of air exposure), seven clutches (1-7) located out of the water and seven clutches (8-14) located in the water were collected and

transported to the laboratory. We covered clutches with dechlorinated water in plastic containers (40 x 20 x 12 cm) until hatching, which finished on 16 April. On 19 April (after at least 31 days of exposure), the last three clutches that were out of the water and that remained in the field were transported to the laboratory. In the last three clutches that remained immersed in the pond, living embryos had already hatched when clutches were collected. Clutches that were out of the water for 31 days were also covered with dechlorinated water in plastic containers (40 x 20 x 12 cm) until hatching, which finished on 25 April. In the laboratory, clutches were maintained at a constant temperature of approximately 15°C. We recorded the number of dead embryos and hatchlings in each container. Because there were empty egg capsules in the outer portions of the jelly matrices when collected, we could not estimate embryo survival rates for whole clutches. Instead, we calculated the survival rate of embryos based on the number that occurred in the egg masses when they were collected and then compared among treatments. For all larvae, we recorded the day of hatching, total length (TL) and Harrison stage at hatching. To record total length and Harrison stage, we located hatchlings in small Petri dishes and observed them using a dissecting microscope. Total length was recorded with a calliper to the nearest of 0.1 mm.

LABORATORY EXPERIMENTS

We also conducted two experiments in the laboratory, to determine whether differences in embryonic development and survival existed between clutches located either in or out of water. We simulated the two situations that we observed in the field on clutches that became naturally exposed to air after water recession. We used 12 clutches of *A. gracile* from a pond that was adjacent to the original pond. Embryos were at Harrison stages 32-36 at the start of the experiments. Immediately before we began the experiments, clutches were cut in half and embryos with damaged perivitelline membranes were removed. Each half-clutch was placed in a plastic container (28 x 18 x 12 cm), and assigned to one of two treatments: air or water. In that way, we eliminated in the statistical analysis the variability due to differences among clutches. Stranded clutch halves in experiment 1 (clutches L 1-6) were placed on a solid plastic base 2 cm high. Suspended clutch halves in experiment 2 (clutches L 7-12) were placed on a metallic grille. The bottom of the grille was 5 cm above the bottom of the container and larvae could pass through the grille. In both experiments, containers with clutches assigned to the "water" treatment were filled to a depth of 10 cm with dechlorinated water and clutches were completely submerged. Containers with clutches assigned to the "air" treatment, were filled to a depth of 1 cm with dechlorinated water to permit hatchling survival after they dropped from the clutch to the bottom of the container. In the "air" treatment, clutches had no contact with water and were

completely exposed to air. Water was replaced weekly and was not artificially aerated. Containers had no lids. Experiments were conducted at a constant laboratory temperature (15°C) and clutch temperature was recorded in the gelatinous surface of every egg mass.

We counted the number of living embryos in every egg mass at the beginning of the experiment. At the end of the experiment, we recorded the numbers of dead embryos and hatchlings in each container. Survival rate was calculated for each container as the ratio of the number of hatchlings to the initial number of living embryos. For all larvae, we recorded day of hatching, total length (TL), and Harrison stage at hatching. To record total length and Harrison stage, we located hatchlings in small Petri dishes and measured them under a dissecting microscope. Total length was recorded with a calliper to the nearest of 0.1 mm. After completion of the study, we released all larvae in the ponds from which they were collected.

DATA ANALYSIS

To compare day at hatching, hatchling stage, and size among treatments, we used average values for every clutch in field observations and half-clutch in the laboratory experiments. To analyse the overall effect of air exposure on embryos in the field (three treatments: 20 days in the water, 20 days out of water and 31 days out of water) we used One-way Multivariate Analysis of Variance (MANOVA). Dependent variables were: ratio of number of hatchlings to total number of embryos after collection, mean day at hatching, mean total length and mean Harrison stage at hatching. We also used One-way Univariate Analysis of Variance (ANOVA) to analyse the differences among treatments in the four variables. In laboratory experiments we used One-way MANOVA for the overall comparison (survival rate, mean day at hatching, mean total length and mean Harrison stage) and paired *t*-tests for univariate comparisons. To comply with normality assumptions, we arcsine-transformed values of the ratios of number of hatchlings to total number of embryos from the field observations and the survival rates from experiments 1 and 2.

RESULTS

CLUTCHES IN THE FIELD

There was no overall effect of air exposure on embryonic development (Table 1). On average ($\pm$ 1SE), 83.1 $\pm$ 0.6% of the hatchlings whose clutches were in water for 20 days hatched during the first day after collection, compared to 90.9 $\pm$ 0.5% of hatchlings that were out of water for 20 days and 96.6 $\pm$ 0.3% of hatchlings that were out of water for 31 days. There were no significant differences in the average day at hatching between these three groups of clutches (ANOVA, $F=1.04$, $P=0.376$), and consequently, laboratory conditions did not influence embryonic development of clutches exposed to the different treatments.

TABLE 1. Results of 3 MANOVAs that analyse the overall effect of exposure to air during embryonic development on *A. gracile* hatchlings (survival and day, size and Harrison stage at hatching). In the laboratory, clutches were stranded in experiment 1 and suspended in experiment 2.

	Effect	df	F	P
Field obs.	Residual	4,11	37966.41	<0.001
	Treatment	8,22	1.00	0.461
Expt. 1	Residual	4,7	25702.13	<0.001
	Treatment	4,7	2.670	0.122
Expt. 2	Residual	4,7	65072.28	<0.001
	Treatment	4,7	2.51	0.136

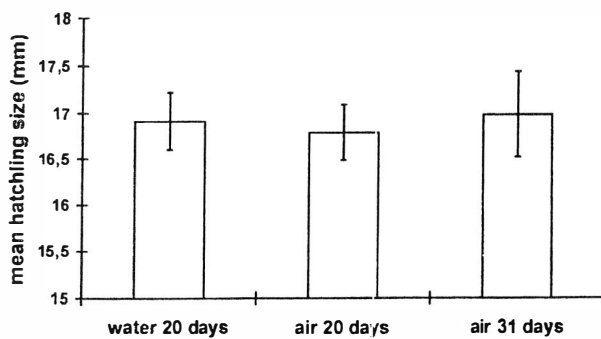


FIG. 1. Mean $\pm$ 1 SE total length of newly hatched *A. gracile* larvae from clutches in (water) and out (air) of water for at least 20 or 31 days in the field. ANOVA: $F=0.181$, $P=0.836$, $df=2, 14$.

The average number of hatchlings, average number of dead embryos and mean ratio of the number of hatchlings to the total number of embryos after collection did not differ among the three groups of clutches (Table 2). There was no difference in average body size (Fig. 1) or in average stage at hatching (Fig. 2) among clutches located in and out of the water for 20 and 31 days.

LABORATORY EXPERIMENT 1: STRANDED CLUTCHES

The experiment began on 23 March and hatching occurred from 25 March until 13 April. In clutches exposed to air, larvae hatched on average after 7 days (range = 2–21 days) of exposure. There was no difference in clutch temperature between treatments (Student t -test: $t=0.703$, $P=0.498$). MANOVA showed no over-

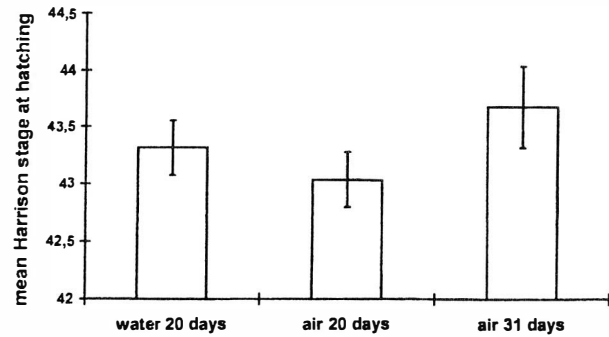


FIG. 2. Mean $\pm$ 1 SE Harrison stage at hatching of *A. gracile* larvae from clutches located in water or in air, in the field. ANOVA: $F=2.564$, $P=0.112$, $df=2, 14$.

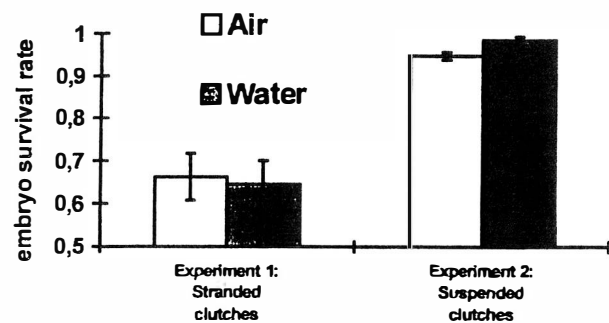


FIG. 3. Mean $\pm$ 1 SE survival rate of *A. gracile* clutch halves located in (water) and out (air) of water from Harrison stages 32–36 in the laboratory. Left: stranded clutches (paired t -test: $t=0.145$, $P=0.891$, $df=5$). Right: suspended clutches (paired t -test: $t=2.52$, $P=0.053$, $df=5$). NS= $P>0.05$.

all significant difference among treatments (Table 1). Embryo survivorship (Fig. 3), and mean day (paired t -test: $t=2.477$, $P=0.056$) and mean Harrison stage at hatching (paired t -test: $t=2.156$, $P=0.084$) were not affected by air exposure. Mean hatchling size was slightly smaller for exposed egg masses than for submerged egg masses (paired t -test: $t=2.647$, $P=0.046$).

LABORATORY EXPERIMENT 2: SUSPENDED CLUTCHES

This experiment began on 26 March and hatching occurred from 2 April until 17 April. Larvae hatched on average after 11.3 days (range = 5–22 days) of exposure to air. There was no difference in clutch temperature between treatments (Student t -test: $t=1.519$, $P=0.166$). MANOVA showed no overall significant difference among treatments (Table 1). Embryo survivorship (Fig.

TABLE 2. Average ($\pm$ 1 SE) number of hatchlings and dead embryos, and average ($\pm$ 1 SE) ratio of number of hatchlings to total number of embryos that remained in clutches of *A. gracile* located in or out of water in the field for at least 20 and 31 days. NS= $P>0.05$. 20d-IN = clutches located in water for 20 days; 20d-OUT = clutches located out of water for 20 days; 31d-OUT = clutches located out of water for 31 days.

	20d-IN	20d-OUT	31d-OUT	$F_{2,14}$
No. clutches	7	7	3	
No. hatchlings	45.6 $\pm$ 6.62	46.7 $\pm$ 6.75	28.3 $\pm$ 10.11	1.67 NS
No. dead	17.1 $\pm$ 4.69	8.6 $\pm$ 4.39	2.7 $\pm$ 7.16	1.29 NS
Ratio	0.77 $\pm$ 0.05	0.85 $\pm$ 0.06	0.88 $\pm$ 0.07	0.38 NS

3), and mean day (paired t -test: $t=0.375$, $P=0.723$), mean size (paired t -test: $t=0.619$, $P=0.563$) and mean Harrison stage at hatching (paired t -test: $t=0.034$, $P=0.974$) were not affected by air exposure.

DISCUSSION

Several sources of amphibian embryo mortality have been reported, including freezing, low pH, UV-radiation, pollutants, pathogens and predation (Berrill *et al.*, 1994; Blaustein *et al.*, 1994, 1995; Harte & Hoffman, 1989; Kiesecker & Blaustein, 1995). Moreover, it seems obvious that desiccation at oviposition sites can be a major cause of embryo death in aquatic breeding amphibians. Air exposure in the field could expose clutches to increased risk of dehydration (air, sun, wind), freezing, mechanical damage and higher exposure to UV-B radiation. Forester & Lykens (1988) pointed out that communal egg-laying behaviour could protect some wood frog, *Rana sylvatica*, embryos from desiccation as ponds dry up. In laboratory experiments, they observed an exponential egg mortality rate for terrestrial exposure, but 11% of eggs were alive after 10 days and 4% survived for as long as 14 days. This relatively long survivorship was attributed to the protection of the viscous coating over the surface of the egg mass (Forester & Lykens, 1988). Nyman (1987) also observed that aggregative egg deposition in *A. maculatum* provided protection from desiccation to aquatic clutches exposed to the air. *A. maculatum* egg masses that were individually deposited masses or were in smaller aggregates did not survive an air exposure period of 35 days. However, *A. maculatum* eggs in the inner protected masses of the largest aggregates (40-80 masses) survived during that period (Nyman, 1987).

Our field observations of *A. gracile* indicated that embryos of individually deposited masses can survive inside the jelly matrix out of the water for at least 30 days. We observed no significant effects on size or stage at hatching due to terrestrial exposure of clutches in the field. In the laboratory, air exposure had no significant effect on embryo survivorship, day of hatching or developmental stage of single clutch halves. We only found a slight effect on stranded clutches that were exposed to air. In these clutches, hatchlings were smaller than those from clutches located in the water. A significant decrease of jelly-mass volume could have led to slight differences between treatments. The decrease of mass volume could have occurred as a result of the halving of clutches in the laboratory experiments. However, our field observations indicate that clutch volume reduction was not substantial in clutches exposed to air. Perhaps the outer jelly envelope provides most of the protection from desiccation. More than one *A. gracile* female may deposit a clutch on the same branch, but in general, clutches are not deposited communally. However, the relatively thick jelly matrix may allow *A. gracile* embryos to avoid water loss and survive drought or temporal water recession.

Clutches are usually attached to rigid branches or sticks and suspended in the water column (Nussbaum *et al.*, 1983). When the water level decreases, clutches can remain suspended above the water or can become stranded on the soil. If larvae from suspended clutches hatch in the air, they could easily fall into the water and survive. Hatchlings from stranded clutches may not have access to water unless rainfall raises the water level before the embryos die.

It will be of interest to discover which other species have eggs resistant to desiccation. This capacity of clutches to survive desiccation is probably related, among other factors, to the structure and composition of the clutch jelly matrix. Many amphibians protect their eggs with gelatinous matrices, but the hardness and consistency of these matrices is very variable (Duellman & Trueb, 1994). Thus, many amphibian species having eggs surrounded by a jelly matrix may not survive prolonged exposure to air. For example, *Rana aurora* eggs laid in the same study pond at the same time, had 100% mortality during the first three days of exposure to air.

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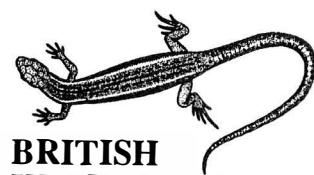
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